LIBRARY
P 171917
OF MICH.
RARY

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

VOL. XXXIII SEPTEMBER, 1917 No. 3

CONTENTS

		PAGE
LILLIE, RALPH S.	*The Formation of Structures Resembling Organic Growths by Means of Electrolytic Local Action in Metals, and the General Physiological Significance and Control of this Type of Action*	135
LOEB, LEO	*The Concrescence of Follicles in the Hypotypical Orary*	187
COWDRY, N. H.	*Comparison of Mitochondria in Plant and Animal Cells*	196

PUBLISHED MONTHLY BY THE
MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

AGENT FOR GREAT BRITAIN
WILLIAM WESLEY & SON
28 Essex Street, Strand
London, W. C.

AGENT FOR GERMANY
R. FRIEDLÄNDER & SOHN
Berlin N. W.
Carlstrasse, 11

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second-class matter under Act of Congress of July 16, 1894.

Editorial Staff

E. G. Conklin—*Princeton University.*

Jacques Loeb—*The Rockefeller Institute for Medical Research*

George T. Moore—*The Missouri Botanic Garden.*

T. H. Morgan—*Columbia University.*

W. M. Wheeler—*Harvard University.*

E. B. Wilson—*Columbia University.*

Managing Editor

Frank R. Lillie—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor, the University of Chicago, Sept. 15th to June 15th, or Woods Hole, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa

Vol. XXXIII. *September, 1917.* *No. 3*

BIOLOGICAL BULLETIN

THE FORMATION OF STRUCTURES RESEMBLING ORGANIC GROWTHS BY MEANS OF ELECTROLYTIC LOCAL ACTION IN METALS, AND THE GENERAL PHYSIOLOGICAL SIGNIFICANCE AND CONTROL OF THIS TYPE OF ACTION.[1]

RALPH S. LILLIE.

I. Introduction.

In a recent paper on the nature of the physico-chemical processes underlying the conduction of stimuli in living cells[2] I have called attention to the existence of various significant parallels between the transmission of the effects of local chemical or other alteration in metals and the transmission of physiological influence in organisms. These parallels suggest that processes of a kind related to electrolysis may be concerned in the physiological type of transmission, since such local action in metals (*e. g.*, rusting in iron) is now recognized as a phenomenon of electrolysis, due to the formation of local electric couples between adjoining regions of the surface which differ in composition or solution-tension. In particular the rapidity with which the chemical and other effects at the immediate site of stimulation are transmitted to remote resting regions, in rapidly conducting tissues like nerve, indicates that any process involving an actual transfer of material from active to inactive regions cannot possibly form the basis of the transmission. Some influence of an essentially different kind is indicated; and the type of phenomenon long known to electrochemists as "chemical action at a distance" [3]—in which a chemical change at one elec-

[1] From the Laboratory of General Physiology, Clark University.

[2] *Amer. Journ. Physiol.*, 1916, Vol. 41, p. 126.

[3] *Cf.* Ostwald's article, "Chemische Fernwirkung," *Zeitschr. physik. Chem.*, 1891, Vol. 9, p. 540.

trode of an electrolytic cell instantly calls forth a corresponding change at the other electrode—seems to furnish the closest physico-chemical analogy. In this chemical "distance-action" the transmission is directly dependent upon electrolysis—*i. e.*, chemical change at the contact of electrode and electrolyte, conditioned by the passing of an electric current between the electrodes. The possibility that a process of essentially this type may occur in living cells is not so remote as might seem at first sight, even though conductors of the first class are not present. In many respects the conditions at the boundary between the living cell and its medium are similar to those at the boundary between a metallic electrode and the adjoining electrolyte. Cell-surfaces are electrically polarized; this polarization is subject to change under a variety of conditions; hence local circuits readily arise in cells and nerve-fibers as a result of stimulation or local alteration or injury. These circuits resemble in various essential respects those arising when dissimilar metals in metallic connection are placed in contact with an electrolyte (the usual battery arrangement); thus (1) the potentials can be summed,[1] (2) the potentials vary with the concentration of the electrolyte in essentially the same manner as in the case of metallic electrodes,[2] and (3) a marked degree of polarizability is shown. The inference that chemical changes of the nature of electrolysis may occur where currents enter and leave the cell-surface would seem to be justified by these resemblances. The existence of such a condition would go far to explain many hitherto obscure types of physiological transmission, besides the conduction of stimuli—for example, the transmission of formative or integrative influence in the phenomena of regeneration, form-regulation, growth and development. The general influence of electric currents upon cellular processes would also appear in a clearer light; it is well known that formative and other metabolic processes, as well as activities like contraction and nerve-conduction, are profoundly influenced by such currents. There are also grounds for believing that in the restoration of the normal resting condition of cells, after

[1] *Cf.* Brünings, *Pflüger's Archiv*, 1903, Vol. 98, p. 241.

[2] Macdonald, *Proc. Roy. Soc.*, 1900, Vol. 67, p. 325. *Cf.* also Loeb and Beutner, *Biochem. Zeitschr.*, 1912, Vol. 41, p. 3.

any kind of activity involving breakdown of material, a process of chemical and structural reconstitution or reorganization takes place, and that in this process electrical factors play an important part.[1] Finally it should be noted that the physiological effects at anode and cathode are typically contrasted in a manner which inevitably recalls the contrast between the two electrodes in electrolysis; and while this resemblance does not prove that processes of electrolysis enter in producing the physiological effect—since the current may have other kinds of polar action—it undoubtedly favors such an interpretation.

The similarity of certain types of electrolytic deposit to vegetative growths has long attracted the attention of chemists. This is especially the case with the so-called lead-trees, tin-trees, etc., obtained when salts of these metals undergo spontaneous electrolysis in contact with baser metals; thus when a piece of zinc is suspended in a solution of tin chloride or lead acetate the metal of the salt is deposited upon the zinc in a tree-like branching form; this metallic deposit grows outward through the solution because each portion of metal as deposited forms a cathodic surface which itself becomes the site of a chemical change similar to that at the original surface of deposition. An analogy to organic growth is evident here; in both processes certain substances are selectively removed from solution, chemically transformed, and deposited to form a definite type of structure. A further resemblance between such a process of electrolytic accretion and true organic growth is that in electrolysis material may be deposited from solutions of *all* degrees of concentration. In this respect the extension of a metallic cathode in a solution of its salt presents a closer analogy to organic growth than does the increase in size of a crystal in a supersaturated solution, since in organic growth the abstraction of food-substances from solution and their transformation into structural material or living protoplasm may also take place from very dilute solution.

Structures having a remarkably close superficial resemblance to plant growths and other cell-structures are also formed under certain conditions (partly described below) from metals under-

[1] *Cf.* my recent paper in *Amer. Journ. Physiol.*, 1917, Vol. 43, p. 43; see pp. 56–7.

going local chemical or electrolytic change in which insoluble precipitates are formed. The rusting of iron is an example of this general type of phenomenon, although here the deposits of oxide, beyond showing a tendency to spread, *i. e.*, to act as centers for the deposit of new oxide, do not as a rule show structural peculiarities definitely suggesting an organic type of growth. Certain other inorganic precipitates, however, such as the ferricyanides of the heavy metals, especially when formed in the presence of protective colloids like gelatine and egg-albumin, may be deposited in a filamentous or vesicular form and give rise to structures which are strikingly similar, both in morphological constitution and in the conditions of their formation, to definite cellular or other organic growths. In the present paper I propose to describe a number of methods by which these structures may be readily formed; and also certain methods of accelerating or inhibiting their rate of formation, which appear to be closely analogous in their fundamental features to the conditions controlling growth-processes in plants and animals. Since these artificial growth-structures are beyond any question produced by local processes of electrolysis, it seems justifiable to regard the various parallels described below as affording strong support to the above view that processes of a similar nature are intimately concerned in the formation and deposition of structural and other materials in living organisms.

II. Experimental.

The formation of filamentous growths from an electrolytically formed precipitate may be most readily demonstrated as follows. A small piece of ordinary iron, *e. g.*, a nail or tack or piece of wire, is placed in a 2 per cent. egg-albumin solution (fresh egg-white diluted six times and filtered) containing 2 per cent. potassium ferricyanide (K_3FeCy_6). Within a few seconds the blue precipitate of ferrous ferricyanide is seen to form locally at various spots on the surface of the metal; the precipitate is deposited chiefly in the form of slender filaments which rapidly increase in number and length until within fifteen to thirty minutes the entire surface of the metal is covered with a blue-green filamentous hypha-like growth. After a few hours some

of the thicker filaments may be several centimeters long, usually extending in a straight or sinuous course over the surface of the solution. They are hollow, unbranched, of smooth contour, and exhibit considerable coherence and flexibility; these properties, however, as well as the rate of growth, vary with the content of the solution in albumin and ferricyanide. The rate of formation also varies with the character of the metal, being in general slower with pure than impure specimens of iron; it is also greatly influenced by the contact of other metals, as will be described in detail below.

The actual process of formation presents a striking spectacle under low powers of the microscope. Within a few seconds the precipitate appears upon the surface of the metal in scattered regions, at first as minute rounded vesicles each enclosed by a thin precipitation-membrane; slender cilia-like filaments then push out rapidly into the solution; these steadily elongate, many reaching in a few seconds a length of 50 microns or more. In most cases growth ceases abruptly at a length of a small fraction of a millimeter; this is usually the case with the more slender formations; the broader filaments may continue to elongate by terminal growth until they reach in many cases a length of several centimeters. At their earliest appearance the filaments strongly suggest organic growths like cilia, and what is still more remarkable is the fact that they often exhibit trembling or wavy or in some cases quite regularly rhythmical to-and-fro vibratory movements. This phenomenon is also observed with the similar filamentous growths of zinc and copper ferricyanide, especially the latter, and will be considered further below. Since in living cells cilia typically arise from locally modified regions of the surface protoplasm ("basal bodies") which serve as centers of rhythmical activity as well as of growth, this behavior of newly formed precipitation-filaments increases the impression that the conditions of formation of these artificial structures are of the same fundamental nature as those of actual cell-structures.

Other metals which form insoluble ferricyanides may give rise in the above solution to filaments of a similar type, differing characteristically in their structure, appearance, and rate of formation from those of iron ferricyanide. Small pieces of zinc

form highly characteristic structures of this kind, especially if in contact with carbon or a nobler metal (Ag, Hg, Pt, Cu, Fe). Pieces of pure zinc, without other metal, form filaments in the above solution much more slowly than iron, presumably because of the more homogeneous structure of zinc and the relative absence of regions differing sufficiently in solution-tension (and hence in potential) to form efficient local couples; but when in contact with a noble metal the rate of formation is rapid. Copper in contact with platinum or carbon also forms filaments in ferricyanide solutions containing sodium chloride in sufficient quantity; for reasons that will appear below, the presence of this or a similar salt is necessary to the process. On the other hand, certain metals which readily form insoluble ferricyanides do not deposit the precipitate in filamentous form; this is true of aluminium, lead, tin, and (in part) manganese. Pieces of aluminium and lead in contact with copper or platinum wire form precipitates rapidly in the above solution; but the deposit merely collects in the form of more or less coherent or vesicular masses at various regions of the surface without forming filaments. Apparently only those metals whose ferricyanides form semi-permeable precipitation-membranes can form filaments. Under the above conditions tin forms a slight and non-coherent precipitate; with manganese some formation of filaments is usually observed, but most of the precipitate is deposited in amorphous form. The following observations have been made chiefly with filaments of iron, zinc, and copper ferricyanide. The more minute structural details of these formations will not be described at length in the present paper,—both in order to save space and also because the experiments themselves are so simple and the materials so accessible that anyone can readily make these observations for himself. The following description will therefore be confined to the more general features of structure and the essential conditions of formation.

Conditions of Formation of Filaments.—In pure solutions of K_3FeCy_6 (2 to 4 per cent. in distilled water) the formation of precipitate is slight and gradual. Pieces of iron or zinc remain for hours in such a solution without perceptible change. The addition of a little soluble alkali salt, however, *e. g.*, NaCl, at

once leads to a rapid formation of precipitate. The growth of filaments in gelatine or egg-white solutions containing K_3FeCy_6 is in fact dependent upon the inorganic salts associated with the protein. Apparently salts must be present whose anions form soluble compounds with the filament-forming metal; the reason for this requirement will become clear when the process of filament-formation is described in detail. The following experiment shows that the rate of filament-formation varies directly with the proportion of chloride (or similar salt) present in the solution of ferricyanide. A small iron nail (one centimeter long) was placed in each member of a series of solutions of 4 per cent. K_3FeCy_6 containing progressively decreasing proportions of NaCl, viz., 4, 2, 1 per cent., etc., down to 1/128 per cent. An abundant precipitate of iron ferricyanide was formed within ten minutes or less in all solutions containing 1/16 per cent. NaCl or more, and the more rapidly the higher the proportion of NaCl. With 1/128 per cent. NaCl the precipitate formed gradually; while in the entire absence of NaCl none was perceptible after several hours. Similar experiments with pieces of zinc in contact with copper wire gave the same result.

The presence of egg-albumin or gelatine, *i. e.*, an emulsoid colloid capable of having a "protective" action upon finely divided insoluble material, greatly favors the formation of coherent filaments from iron and zinc; with copper this condition is less important. Thus in a solution of 1 per cent. K_3FeCy_6 in distilled water containing a trace of HCl ($n/200$–$n/400$) an iron nail rapidly forms precipitate, but this is largely separated in the form of a loose non-coherent deposit and only partly as filaments. In a similar solution containing 2 per cent. egg-albumin relatively little amorphous precipitate is formed, and the filaments are more numerous, regular, and coherent. Similar conditions hold to an even greater degree for the formation of zinc filaments; in simple aqueous solution the zinc ferricyanide is deposited chiefly in amorphous form or in the form of coarse, brittle, and irregular tubular structures; while with protein present an abundance of slender hypha-like filaments results. This type of phenomenon is undoubtedly of much biological interest. In the absence of the protective colloid the crystals of

precipitate unite as they separate out to form coarser aggregates of relatively slight coherence; while in its presence each particle of precipitate is apparently at once coated with an adsorbed film of modified protein which serves to bind the particles together and favors the formation of delicate and regular structures. In organisms the deposition of inorganic precipitates like calcium carbonate or phosphate in the formation of skeletal structures (bone, shell, etc.) takes place under essentially similar conditions; the particles are thus deposited in finely divided form, and their arrangement to form definite and regular structures is facilitated. This influence of albumin or gelatine upon the character of precipitation-membranes and similar structures has been investigated by Quincke;[1] he also finds that the colloid modifies very essentially the character of the structures formed.

A certain minimal concentration of K_3FeCy_6 is also necessary for the formation of coherent filaments. In lower concentrations the precipitate tends to separate out in non-coherent form, producing irregular or amorphous deposits. Thus in experiments with iron nails placed in a series of solutions containing 2 per cent. egg-albumin and 1 per cent. NaCl, with varying concentrations of K_3FeCy_6, it was found that while from a 0.8 per cent. solution of K_3FeCy_6 the precipitate separated chiefly in filamentous form, from a 0.4 per cent. solution it was deposited only in small part as filaments but chiefly as an amorphous mass; in 0.2 per cent. very few filaments were formed, and none in more dilute solutions. The same general result was found in a second similar series with a lower concentration of NaCl (1/8 per cent.). Similarly while zinc readily forms filaments in an albumin solution containing 0.25 per cent. K_3FeCy_6, in a 0.13 per cent. solution only a few filaments are formed. In order that coherent structures should be formed the concentration of soluble membrane-forming salt must exceed a certain well-defined minimum.

Structure and Manner of Formation of Filaments.—The precise structure of the filaments varies according to the nature of the metal, the presence or absence of protective colloids, and the concentration of the ferricyanide and other salts present. The

[1] Quincke: *Annalen d. Physik*, 1902, 4te F., Vol. 7, p. 646.

larger filaments are typically hollow or tubular; it is only the shorter and slenderer structures that appear to be solid, and even here the case is doubtful, since filaments that appear solid on ordinary examination often show a distinct double contour when examined more closely. The probability is that all filaments are hollow, at least when first formed, and that each consists essentially of a solution containing a salt of the metal and enclosed by a semi-permeable precipitation-membrane. The mode of formation about to be described indicates this clearly. The appearance of the mass of filaments covering a piece of metal that has lain for some time in the solution is strikingly vegetative or fungoid in character. The special character or "habit" of the growth varies with the different metals; in the case of iron and copper the filaments are usually more uniform in appearance and have a smoother contour and straighter course than in the case of zinc; with the latter metal the growths are more irregular and varied and have a characteristic granular appearance due to adhering coarser particles of precipitate. The appearance of such growths is often remarkably hypha-like in character; thus a thin strip of zinc about two centimeters long with a copper wire bound about one end, placed in a watch-glass containing a 2 per cent. solution of K_3FeCy_6 in dilute egg-white, developed in the course of eighteen hours a most complicated feltwork of winding and interlacing hypha-like filaments, covering the whole strip from end to end. Numerous tubular filaments extended to the surface of the solution and along the surface, in some cases for three or four centimeters; many such surface-filaments had undergone curious modifications suggesting efflorescences or spore-capsules, *e. g.*, becoming enlarged at their terminations to form stalked vesicular or bladder-like formations with thin walls. Such a mass of modified filaments irresistibly suggests a vegetative growth.

Study of the mode of formation of these tubular filaments under the microscope reveals a somewhat unexpected complexity of conditions. Elongation is not by basal growth, *i. e.*, by deposition of precipitate at the surface of the metal where the metallic ions enter solution, but is always *terminal*, the tube advancing through the solution by the deposition of precipitate

at its extremity; this process is accompanied by a flow of solution outward through the tube. Filaments of iron ferricyanide show most clearly the nature of the whole process. In a 2 per cent. solution of K_3FeCy_6 in dilute egg-white containing a little NaCl (0.25 per cent.) large hollow filaments grow out rapidly from the metal within a few seconds. Each such filament advances into the solution at an apparently rapid rate (of a millimeter or more per minute) by a uniform motion; its contour is smooth and regular and its shape cylindrical or slightly tapering; it usually follows a straight course, but is easily diverted by encountering obstacles, such as solid particles or other filaments, or by shaking the watch-glass so as to make irregular the deposition of precipitate at the extremity. In a growing filament this extremity is always open, and from the orifice a stream of liquid containing fine suspended particles flows continually outwards for a short distance in advance, suggesting somewhat the smoke emerging from a chimney; this stream of finely divided material leaves behind and on either side a trail of particles through which the growing tubule advances; while at the edges of the open orifice a dense uniform layer of precipitate is continually being deposited in continuation of that already laid down. Growth proceeds rapidly in this manner until some condition arrests the outflow of fluid; this may happen as a direct result of increasing length, the flow through the long capillary tube being slowed more and more by frictional resistance as the length increases, until finally the end is sealed by the precipitate. Evidently a wide tube will grow longer, under these conditions, than a narrow one, and in fact the great majority of filaments cease growth at a length of one or two millimeters or less; it is only the exceptional wider tubes that reach the length of a centimeter or more.

From these facts it is clear that elongation is dependent upon the maintenance of a flow of solution from the metal along the tube. The precipitation at the open end shows that this solution contains a dissolved salt of the metal. Apparently the process takes place somewhat as follows. At the anodic portions of the metallic surface ferrous ions enter solution; these form with the ferricyanide ions of the solution already present a precipitate of

ferrous ferricyanide which is deposited as an incomplete vesicle or ring of semipermeable membrane forming the beginning of the tube; ferrous ions continue to be given off from the iron surface, forming ferrous chloride with the chlorine ions present; the interior of the tube thus contains a solution of ferrous salt which is continually being propelled forward through the open extremity, and there meeting ferricyanide from the external solution forms the precipitate.

The question of what maintains the flow is interesting. One possibility is that each ferrous ion is hydrated, *i. e.*, carries water along with it as it enters solution; water thus flows in the same direction as the ferrous ions, the energy of the flow being derived from the solution-pressure of the iron. Other factors, however, probably enter; it is especially to be noted that the point of origin of each tube represents an anodic area, from which the positive electrical stream enters the solution, reëntering the metal at some cathodic region or regions outside of the tube. An electric current thus passes outward along the tube; and this must cause effects of the kind always found when such a current traverses a system of capillary tubes containing a solution, *e. g.*, a porous partition; these effects are of the kind known as "electrical endosmose";[1] when the substance of the capillary wall is negatively charged the adjacent solution is positive and travels with the positive stream. It is known that iron ferricyanide particles are charged *negatively* in contact with water;[2] hence the layer of solution at the walls of a tube consisting of this material must be *positively* charged and undergo transport in the direction of the positive stream. The flow of solution through the tube is thus probably in large part due to electrical transport by the current of the local circuit. One necessary factor in the process of formation of the tube is the semi-permeability of its wall, which permits water but not salts to penetrate; the solution of ferrous salt thus transported along the tube can

[1] For a description of this phenomenon see the larger textbooks of physics; a good account of the essential conditions is given in Höber's "Physikalische Chemie der Zelle und der Gewebe," 1914, pp. 234 *seq.*

[2] The ferrocyanides and the ferricyanides of the heavy metals form negatively charged particles. The potential-difference of Prussian blue against water is .056 volt. (*Cf.* Burton, "The Physical Properties of Colloidal Solutions," London, 1916, p. 135.)

form precipitate only where it flows out at the open extremity and meets the ferricyanide outside; part of the precipitate is scattered outside the tube, as already described, or remains finely suspended within its interior; but part adheres at the outlet and contributes to the growth of the tube.

According to this conception of the tube-forming process, the rate of flow of fluid along the tube must vary directly with the intensity of the electric current through the tube—*i. e.* (assuming constant E.M.F.), with the electrical conductivity of the solution within the tube. This explains why the rate of growth of the filaments varies directly with the concentration of NaCl (or similar salt) in the ferricyanide solution. The only salt that can remain in solution inside the tube and contribute to the conductivity of its contents must obviously be one that is not precipitated by the ions of the tube-forming metal.

Structure of Filaments.—The structure of a tubule is partly determined by the nature of the material composing it, partly by the conditions under which the material is laid down, such as the size of the anodic area, concentration of salts, presence of protective colloid, external mechanical influences, etc. In general the consistency of the precipitate is coarsest in the case of zinc, and finest in the case of copper; hence tubes of zinc ferricyanide have a coarsely granular aspect and are more irregular in form, while those of iron and copper have smoother contours and a more uniform appearance. There is always considerable variation in the diameters of the tubes formed in any single experiment; in general an increase in the concentration of NaCl in the solution (*i. e.*, more rapid rate of formation) favors the production of wider tubes. Apart from differences in caliber there is little variation in the structure of those tubes which lie entirely below the surface of the solution; typically these are cylindrical or slightly tapering in shape, and follow a more or less tortuous course, varying according to the number and nature of the mechanical obstacles encountered during their formation; zinc filaments show more irregularities of this kind than iron or copper filaments. Those filaments which reach the surface of the solution during their formation undergo various characteristic modifications; thus vesicular or bladder-like thin-walled structures may

be formed, especially in the case of zinc, as above described; several such structures derived from different filaments may fuse and form a compound vesicular or cellular mass. One of the most interesting modifications is of a kind often shown by surface-filaments of iron or copper ferricyanide, consisting in a series of transverse striations resembling those of a striated muscle fiber. A regular ladder-like structure of this kind may extend for a long distance; it is apparently due to the presence of alternately denser and thinner zones in the precipitation-membrane forming the wall of the tube. This structure is found only in the larger filaments running along the surface of the solution, and indicates that the deposition of precipitate takes place in an intermittent manner under these conditions; the regular rhythmical recurrence of a metastable condition of some kind, such as supersaturation—as in the phenomenon of the Liesegang rings[1]—may determine its production, but the precise conditions require further study. Occasionally the finely divided precipitate inside the tube may aggregate to form rounded masses repeated at regular intervals, in a manner suggestive of a row of spores.[2] Filaments of zinc ferricyanide may at times exhibit a regularly constricted or moniliform appearance, but they never form regular cross-striations like those shown by iron and copper filaments; the coarser texture of the precipitate is probably responsible for this difference.

Certain other types of precipitation-structure should also be mentioned briefly.[3] Masses of precipitate of a vesicular or chambered rather than filamentous structure are often laid down at the surface of the metal; this is more frequently the case with zinc than with iron or copper. Apparently in such cases the walls of the first formed vesicles remain intact, and do not rupture to allow the solution of metallic salt to flow out in the continuous

[1] *Cf.* Liesegang, "Beiträge zu einer Kolloidchemie des Lebens," Dresden, 1909. For a more recent study of this phenomenon *cf.* Stansfield, *American Journal of Science*, 1917, Vol. 43, p. 1.

[2] The structure of these tubules resembles closely that of the "osmotic stems" described and pictured by Leduc in his "Mechanism of Life," London, 1911, pp. 141–2.

[3] In Lehmann's "Molekularphysik" various precipitation-formations are compared with different types of organic structure, and much interesting detail is given.

stream necessary for the formation of filaments. Once formed, these precipitation-vesicles may be displaced or detached by the pressure of others formed later, and large groups or heaps of such structures may thus collect. In the case of copper one highly characteristic type of formation, frequently found in solutions of K_3FeCy_6 containing considerable chloride (*e. g.*, 4 per cent. NaCl *plus* 2 per cent. K_3FeCy_6), is a group of slender tapering filaments grouped radially about a central vesicular mass of precipitate, the whole structure suggesting a miniature chestnut-burr.

It should be noted that these various types of structure, together with many others, are produced—as is also the case with the majority of living structures—by combinations of microscopic hollow elements, vesicular or tubular in form, with walls consisting of semi-permeable membranes. The above resemblances to organic growths are undoubtedly dependent upon this fundamental similarity of physico-chemical constitution.

Influence of Contact of other Metals upon Filament-formation.—Before proceeding to describe observations showing the inhibiting and reinforcing influence which the contact of one metal may exert upon the formation of filaments from another, the general theory of this effect ought briefly to be considered. As already explained, the formation and growth of these structures depend upon a process of electrolytic local action or electrolysis; the region from which the filament grows out represents an anodic area, *i. e.*, an area where ions of the membrane-forming metal enter solution; these ions then interact with the ferricyanide ions to form the precipitate; to each such anodic area corresponds a cathodic area (or areas) at some other region of the metallic surface, where the positive stream reënters the metal to complete the circuit; at this area hydrogen ions are deionized and hydrogen gas is freed with the formation of alkali. In the case of iron these areas may readily be demonstrated by the addition of a little phenol phthaleïn to the ferricyanide solution (as in the ferroxyl reagent of Walker);[1] the cathodic regions, which typically remain bright and free from filaments for some time, then

[1] *Cf.* Walker, Cederholm and Bent, *Journ. Amer. Chem. Soc.*, 1907, Vol. 29, p. 1251.

exhibit the characteristic red color of the indicator in alkaline solution. Each filament thus originates from an anodic area in a local electric circuit; the precise mechanism of the formative process has already been considered.

Specimens of ordinary iron always exhibit a large number of local couples of this kind, because of the characteristic lack of electro-chemical homogeneity of this metal. As has frequently been pointed out, this condition explains the susceptibility of iron—especially impure iron—to rust; an iron surface is composed of numerous areas which differ in physical condition and hence in electrolytic solution-tension; areas of higher solution-tension form local anodes, and rusting is a consequence of electrolysis at these local circuits. Iron prepared in as high a state of purity as possible is surprisingly resistant to corrosion.[1] What is true of rusting is true also of the formation of precipitates with electrolytes like ferricyanide; filaments form more slowly from chemically pure iron wire than from pieces of ordinary commercial metal (nails, etc.). Zinc and aluminium, although metals of higher solution-tension than iron, are more readily obtained in a relatively homogeneous condition; hence they resist corrosion better and form precipitate more slowly in ferricyanide solutions.

Such considerations explain the accelerating influence which the contact of a nobler metal, like platinum or copper, has upon the formation of precipitation-filaments from zinc, iron, or copper. The nobler metal (that of lower solution-tension) has a higher potential in contact with the solution than the less noble; hence a circuit is formed in which the latter acts as anode, *i. e.*, enters solution as cations which form precipitate with the ferricyanide. Conversely, a less noble metal, *e. g.*, magnesium or manganese in relation to iron or zinc, *inhibits* the formation of filaments from the membrane-forming metal. Both the reinforcing and the inhibiting influences have very pronounced and evident effects, which are perceptible not only at the immediate region of contact of the two metals, but for a considerable distance, *e. g.*, of several centimeters, beyond this region. The degree of this influence at any point on the surface of the filament-forming

[1] For references to the literature in this field see my paper above cited, *Amer. Journ. Physiol.*, 1916, Vol. 41, p. 126.

metal depends in general upon three chief factors; these are, (1) the nature of the second metal, (2) its distance from the point under consideration, and (3) the electrical conductivity of the solution. The electromotive force of the local circuit depends primarily upon the difference between the specific solution-tensions of the two metals; hence under otherwise equal conditions zinc is less effective than manganese, and manganese than magnesium, in inhibiting the formation of filaments from an iron surface. The other two factors, (2) the distance between the contact of the metals and the point in question, and (3) the electrical conductivity of the solution, determine the resistance of the portion of the local circuit which includes that point; this determines the intensity of the current and hence the rate of local electrolysis at the point. The effect is thus greatest near the region of contact and diminishes as the distance from this region increases.[1]

The actual phenomena may now be illustrated by the description of typical experiments. In order to show the accelerating influence of a nobler metal, zinc is more satisfactory than iron, for the reason that it can more readily be obtained in comparatively pure and homogeneous condition, in which state it forms precipitate in ferricyanide solutions very slowly unless in contact with the other metal. It is difficult to procure non-reactive specimens of iron; pure iron wire of the kind used in standardizing (*e. g.*, Baker's labeled 99.7 per cent. pure) forms filaments rapidly in a 2 per cent. solution of K_3FeCy_6 in dilute egg-white; the rate of formation is accelerated by encircling the iron with copper or platinum wire, but the degree of acceleration is comparatively slight, and the effect is much less striking than when zinc is used.

Such experiments as the following are easy to perform and invariably give striking results. A small strip, *e. g.*, 2 cm. long by 1 mm. wide, cut from a bar of pure zinc, is encircled at one end with a ring of fine copper wire, and placed in a 2 per cent. solution of K_3FeCy_6 in dilute egg-white. Within a minute or two the characteristic thin-walled transparent vesicles appear

[1] *Cf.* the second paper of my recent series on the physical chemistry of the conduction-process, *Amer. Journ. Physiol.*, 1915, Vol. 37, p. 348.

at the surface of the metal, and somewhat later slender filaments begin to grow out; in a short time (half an hour or less) the metal is covered with filaments and vesicular formations from one end to the other. The first appearance of these structures is typically near the contact with the copper; a gradient in the rate of formation is thus apparent, with the most active region near the copper. This accelerating influence of the nobler metal is clearly perceptible at the extremity of the strip, *i. e.*, for a distance of some centimeters from the contact. A similar "control" piece of zinc, without copper, shows only a few irregular and slightly developed filaments at a time when the copper-bound piece is completely covered with a luxuriant hypha-like growth. Platinum and iron wires have the same general effect as copper, only with platinum the accelerating influence is distinctly greater, and with iron less, than with copper. Carbon may also form the substance of the cathodic area; a piece of zinc marked with lead-pencil shows a rapid development of filaments, beginning at the marked area and grading off from there.

Striking experiments may also be performed with copper as the filament-forming metal. In this case a higher concentration of NaCl is required for the rapid formation of filaments; beautiful structures develop after some minutes in a solution containing 4 per cent. K_3FeCy_6 and 4 per cent. NaCl (with or without egg-white). A piece of fine copper wire 3 or 4 cm. long, encircled at one end with a platinum wire, or wound around a piece of carbon, shows a highly characteristic development of filaments; some of these within a few hours may reach a length of several centimeters; such filaments, as already described, often exhibit a very perfect and regular cross-striation where they run along the surface of the solution. A similar piece of copper wire without contact of nobler metal or carbon remains unchanged.

Many variants of this type of experiment will readily suggest themselves. Experiments with plated wires yield instructive results. Pieces of "galvanized" (zinc-plated) iron usually show the first development of filaments of zinc ferricyanide near the cut surface where the iron is exposed, and gradients in the rate of formation extending from this region along the wire are frequent. It should be added, however, that these gradients

are often obscured by irregularities in the rate of action, due presumably to lack of uniformity in the zinc layer (local interruptions, etc.). Platinum wires coated with copper in an electrolytic bath also make interesting demonstrations; such a wire shows no formation of filaments in a solution of K_3FeCy_6 and NaCl if the coating of copper is continuous; but if it is scraped away at any region so as to expose the underlying platinum, the copper near this region soon puts out filaments, and the process extends for a distance of some centimeters from the area of exposure. Such experiments are instructive as exemplifying the nature of the effects that may follow local alteration in the electromotor character of a surface; and they may be used to illustrate by analogy such physiological phenomena as the initiation of growth-processes or regeneration in living organisms by incision, amputation, or similar treatment.

The reverse type of experiment, in which the formation of filaments from one metal is inhibited or prevented by contact with another metal of higher solution-tension, is equally readily performed. Such an effect is illustrated in the just cited experiment with zinc-plated iron wire; the exposed iron surface develops no filaments; their formation is evidently prevented by the adjacent zinc, which forms with the iron a couple in which the zinc is anode; this, it may be added, is the reason why such wires do not rust. The inhibitory influence of zinc upon the formation of ferricyanide filaments from iron may be shown in a more striking manner as follows; a straight piece of thin bright iron wire (*e. g.*, Baker's C. P.) some centimeters long, one end of which is wound about a small strip of zinc, is placed in a 2 per cent. K_3FeCy_6 solution in dilute egg-white. Filaments put forth rapidly from the zinc, especially near the iron, but the iron itself remains perfectly bright and bare, and may show no development of filaments for hours. If then the wire be cut in two by scissors, the part remaining in connection with the zinc remains unchanged, while the isolated part quickly develops the characteristic blue-green filamentous growth of ferrous ferricyanide. Evidently this development had previously been repressed by the influence of the zinc; when the connection is severed the iron reacts as usual. This experiment is even more

striking if a metal of higher solution-tension than zinc is used, such as aluminium, manganese, or magnesium. Magnesium is especially effective, since the ferricyanide of this metal is soluble and hence does not accumulate at the anodal regions and arrest the inhibitory action by polarization; such an iron wire remains bright until the magnesium is completely dissolved; then filaments grow out as usual. Apparently when zinc is used the accumulation of zinc ferricyanide may interrupt contact between the metals or render the resistance of the local circuit too high (*i. e.*, have a polarizing effect), and by degrees filaments form from the iron. In addition to this effect, contact of the zinc ferricyanide precipitate with the iron appears to form local couples in which the iron acts as anode, so that iron in contact locally with this precipitate usually exhibits in course of time a development of blue filaments. This, however, is an incidental effect due to the formation of another type of circuit. Pieces of aluminium or manganese are almost as effective as magnesium in preventing the formation of iron filaments in the above solution. A large variety of experiments of this type may be performed with iron wire. In a similar manner zinc may be used as the filament-forming metal, and the process inhibited by magnesium, aluminium, or manganese.

As already mentioned, this inhibitory influence may be effective for a distance of several centimeters from the region of contact of the two metals, and a gradient in the intensity of the influence is apparent. After the current of the circuit has decreased, as the result of the accumulation of precipitate or other polarization, an iron wire with zinc connection often begins to send out filaments; these tend to be first formed at the region farthest removed from the zinc. Irregularities, however, are frequently observed in such experiments, due no doubt to local irregularities in the composition and physical condition of the iron. The biological analogies to these effects will be considered later.

An interesting type of effect is seen when an iron wire 3 or 4 cm. long is in contact at one end with a piece of zinc and at the other with copper or platinum. With this arrangement gradients in the formation of iron filaments are very distinct; at first these

filaments are formed only in the immediate neighborhood of the copper and are entirely absent near the zinc. Copper and zinc influence the reaction of the iron wire with the solution in opposite manners, the former promoting the formation of iron filaments, the latter inhibiting this process. The zinc and the copper, although separated by the length of iron wire, can be shown to modify each other's action; thus filaments grow out from the zinc under the influence of the iron, but more slowly than in the absence of the copper; similarly the growth of filaments from the iron near the copper is much slower than in the absence of the zinc. The effect is as if the formation of filaments from the zinc inhibits or holds in check the formation of filaments from the iron, and *vice versa*. This mutual interference is obviously due to the intersection of the electrical currents from the two circuits, and resulting compensation-effects. Severing the iron wire between the copper and the zinc removes this influence, with the result that then filaments are put forth rapidly from both iron and zinc; *i. e.*, the process at either filament-forming metal is released from a certain constraint, due to the polarizing influence of the electrical current from the other circuit; it then proceeds more rapidly than before. Physiological analogies to this type of effect readily suggest themselves; the development of shoots in one region of a plant stem inhibits their development in adjoining regions; removal of a rapidly growing shoot may thus cause buds to develop from the axils and elsewhere, which otherwise would have remained dormant.[1] Similar phenomena are seen in hydroids and other animals, and will be discussed more fully below. Physiological "dominance" of this type is often simulated in a suggestive manner by experiments of the above kind. For example, a thin strip of zinc 3 cm. long was in contact at one end with a piece of pure iron wire and at the other end with a piece of magnesium. A well-marked gradient was shown; near the iron filaments were rapidly formed from the zinc, but near the magnesium slightly and slowly. It was noteworthy that the piece of iron wire gradually formed filaments in spite of the contact with the zinc; *i. e.*, the presence of the magnesium

[1] For numerous examples *cf.* McCallum, "Regeneration in Plants," *Botanical Gazette*, 1905, Vol. 40, pp. 97, 241.

inhibits the protective influence of the zinc upon the iron,—evidently because the zinc forms the cathode in the zinc-magnesium couple, the current from which opposes and partly compensates that from the zinc-iron couple. In another experiment a similar strip of zinc was in contact with platinum wire at one end and with magnesium at the other; in this case the zinc remained free from filaments for a long time; after 18 hours a dense growth of filaments had developed near the platinum; toward the magnesium the growth decreased, and near the contact with this metal the zinc was entirely free from filaments. In a similar manner the contact of iron or zinc with a platinum-bound copper wire inhibits the formation of filaments from the copper. A piece of copper wire 4 cm. long, encircled at one end with platinum wire, was placed in a solution containing 4 per cent. K_3FeCy_6 and 4 per cent. NaCl; filaments and bur-like tufts of copper ferricyanide appeared rapidly. But a similar piece of platinum-bound copper which was placed in contact at the other end with an iron wire showed only slight and gradual formation of precipitate; under these conditions filaments form rapidly from the *iron*, and their formation apparently has the effect of inhibiting their development from the copper. What really happens is that the current of the copper-iron circuit is opposed in direction to that of the copper-platinum circuit and compensates the latter.

Attention should also be called to a type of experiment in which the two precipitate-forming metals are not in direct contact with each other but are connected by a conductor which itself undergoes no chemical change, like platinum, carbon, or copper. For example, a piece of iron and a piece of zinc connected by a stretch of copper wire 1 cm. long were placed in 2 per cent. K_3FeCy_6 in dilute egg-white; the zinc immediately formed filaments, but not the iron. Similar pieces of iron and zinc, each in contact with a separate piece of copper, both form filaments rapidly, as already described; but when the two are in contact with the same conductor the zinc prevents the formation of filaments from the iron in the same manner (though to a less degree) as when the metals are in direct contact. It is further to be noted that under these conditions the iron, though not

itself forming filaments, retards or partly inhibits their development from the zinc; *i. e.*, zinc in contact with copper alone forms filaments more quickly than when in contact with a copper wire which is itself in contact with iron. This mutual influence is greatest when the two metals are close together; when they are separated by a sufficient stretch of conducting wire the effect becomes inappreciable. Thus with a distance of 3 cm. between the zinc and the iron, the latter metal, as well as the zinc, formed filaments, but more slowly than in the absence of the zinc; the influence of the zinc was perceptible, but insufficient to suppress entirely the action at the iron. Increasing the distance of separation still further renders the influence inappreciable. This experiment recalls such physiological phenomena as the decrease of growth-inhibiting dominance with distance.[1] Experiments with platinum as the connecting metal give similar results; only here, because of the nobler character of the platinum, the distance between the zinc and the iron must be less than when copper wire is used, if the formation of filaments from the iron is to be entirely suppressed. In other words, both zinc and iron have a stronger tendency to form filaments when in contact with platinum than when in contact with copper; hence in order to counteract this tendency in the iron its distance from the zinc must be less.

It will be evident that effects of the above kind have many close biological analogies.[2] Increased physiological or metabolic activity at one region of a living organism often results in the decrease or cessation of activity at another, usually adjoining, region. Perhaps the best known instances are seen in various phenomena of growth and regeneration to which attention already has been called; the reciprocal inhibition of antagonistic neurones in the central nervous system is probably a phenomenon of the same essential type. There is also good reason for associating with this class of phenomena such regular and characteristic physiological processes as the automatic initiation and arrest of activity at any region of a conducting element (*e. g.*,

[1] *Cf.* Child, "Senescence and Rejuvenescence" (especially Chapter 9), and "Individuality in Organisms," for a full account of these phenomena (University of Chicago Press, 1915).

[2] See Section III. below, page 171.

nerve fiber) as the advancing wave of excitation reaches and passes that point.[1] In the case of these various vital phenomena it seems highly probable, from the degee and nature of the resemblances which they bear to the simple physico-chemical phenomena described in this section, that the essential determining factors are also electrical currents between adjoining cells or cell-regions. These, according to their direction, local density, and intensity, may initiate, inhibit, or reinforce processes at various points along the path of the current.

It may sometimes be desirable to demonstrate still further the electrical nature of these inhibitory and accelerating influences. This may be done most readily by passing the current from a battery, connected with a rheocord, through the ferricyanide solution, using iron wire as electrodes. The formation of filaments at the anode is promoted and at the cathode is prevented, to a degree which varies with the potential between the electrodes; this potential may be changed at will by shifting the position of the slider.

III. Biological Comparison.

In comparing phenomena of the above described kind with physiological phenomena it is obvious that careful distinction must be made between resemblances that are superficial and resemblances that are based upon a fundamental identity in the nature of the determining conditions. It need scarcely be pointed out that in the specific character of the structure-forming chemical reactions there is no resemblance between the two processes under comparison. The complex and imperfectly understood metabolic transformations in living cells are of a kind entirely different from the simple cross-decompositions and precipitations of the above experiments. The resemblances relate to the conditions under which the chemical reactions take place, to the type of structure formed, and to the mode of transmission of chemical influence from one region to another region which is connected with the first only through the medium of an electric conductor. Chemical action at a distance is a phenomenon universally met with in organisms; and the attempts to explain

[1] *Amer. Journ. Physiol.*, 1916, Vol. 41, see pp. 134–5.

this action on the basis of transport of actual materials—through the flow of sap, blood, or other circulating medium—are in many cases patently inadequate. Consider—to take an extreme instance—the transmission of chemical influence from the brain to a secreting gland-cell; here obviously the problem of the nature of physiological conduction enters; and it is more especially this problem—the nature of transmission of physiological influence where no direct transmission of material is possible—upon which experiments of the above kind appear to throw light. The problem is plainly a most fundamental one, since physiological coördinations of the most varied nature depend upon the rapid conduction of chemical influence between different parts of the organism. The integration of the whole complex system of structures and chemical reactions into a unified and regularly acting living organism probably depends more essentially upon transmission of this type than upon any other single process.

The transmission of formative influences, sometimes of a growth-inhibiting, sometimes of a growth-promoting kind, from one region of the organism to another is a well-known phenomenon in both animals and plants. Usually it has been explained either as due to the production and transport of special materials (organ-forming substances, hormones, etc.), or as due to changes in the distribution of materials already present (changes in the flow of water in plants, increased availability of nutritive substances to one organ when another organ is removed, etc.). In animals a special rôle has at times been assigned to nervous influences; the nervous system[1] or sense-organs[2] have been shown in some cases to influence the rate of regeneration, *i. e.*, a transmissive instead of a transportative means of control[3] has been recognized; but in plants explanations based upon the assumed existence of form-determining

[1] This influence is seen (*e. g.*) in Herbst's experiments in which the regeneration of the eye in Crustacea was found to depend on the presence of the optic ganglion; also in planarians and other forms.

[2] The presence of the marginal sense organs in regenerating pieces of the medusa *Cassiopea* favors regeneration, according to the recent observations of Cary, *Journ. Exper. Zool.*, 1916, Vol. 21, p. 1.

[3] For a discussion of the general nature of this influence see Child's "Individuality in Organisms," especially Chapter 6.

substances have been most generally employed (Sachs and others).[1]

Among the various phenomena of organic growth and development, regenerative processes seem most evidently open to interpretation in the light of the foregoing experiments. In the regenerating organism growth or development is initiated as a result of the removal of one of its parts, the usual result being the restoration of the missing part or its equivalent. In many cases the part removed is replaced by growth at the cut surface; in other cases the remaining portion of the organism undergoes a more or less extensive reorganization, called by Morgan "morphallaxis"; in still others, especially in plants, removal of an organ initiates the development of a similar organ from another locality, *e. g.*, when the removal of the growing tip induces the development of lateral branches from axillæ or other regions at a distance.

The first class of cases, where growth at the cut surface restores the missing part, may be compared with the case of a membrane-forming metal in a ferricyanide solution, *e. g.*, a piece of iron wire in which the filamentous or membranous growth has accumulated to a degree sufficient to insulate the metallic surface from the surrounding solution, *i. e.*, to arrest the flow of current between anodic and cathodic regions by the interposition of the non-conducting coating of precipitate. If then a cut be made exposing a fresh surface of metal, the formation of precipitate is at once resumed and continues until a new state of equilibrium is established; the accumulation of electrolytically formed structure will then be approximately the same as before. The whole structural system, consisting of the metal with its outer coating of precipitate, will exhibit essentially the same constitution as before. Again in the case of a strip of zinc encircled by a copper wire, as in the experiments described above, the zinc forms the anode of the couple, and its surface is soon covered by precipitation-structures, whose accumulation progressively retards the flow of current and with it the structure-forming electrolysis. When the fresh surface of zinc is exposed, the formation of a new filamentous coating follows simply from the reëstablish-

[1] For an experimental examination and critique of this type of hypothesis, *cf.* the paper of McCallum above cited.

ment of the electrical circuit. As the current flows, structural material is formed and deposited at the anode, until its accumulation increases the resistance of the circuit and checks the current and hence the electrolysis which produces the structure. Similarly in the case of a regeneration-process where proliferation starts at a cut surface at which previously quiescent cells have been exposed, it is possible that the condition of quiescence is due to insulation by the surrounding cells and other tissue-elements; if these are removed the flow of an electrical current and the formation of new structure are enabled to take place.

The case of morphallaxis is more complex, and is difficult to simulate by a model of the above kind. In this case constructive processes at one area are accompanied by regressive processes at another area; *i. e.*, growth at one region forms the condition of inhibition of growth or of positive regression at other regions; the whole mass of organized living material is thus worked over or reconstituted until a second equilibrium is reached; the character and distribution of structural material is then similar to that existing before mutilation took place. The conditioning of inhibitory processes at one region by active processes at another, and *vice versa*, is a frequent phenomenon in organisms; and analogies are afforded by the experiments cited in the preceding section. In general, in any electrolytic couple the processes at the anode are of an oxidative, those at the cathode of a reducing nature; and it is not difficult to understand how in a living organism increased chemical activity of a certain kind at one region (*e. g.*, oxidation) should result in decreased activity (or activity of the reverse type) at another region related to the first in the manner in which the electrodes of a circuit are related to each other. The inhibition of filament-formation at iron surfaces by the contact of a baser metal illustrates an essentially similar condition. The constant form-relations of an organism or of its parts may thus represent a condition where a complex system or combination of electrolytic processes, interrelated as above, mutually equilibrate one another, such equilibration involving a certain constancy in the relative positions, dimensions, and form-characters of the chief structural elements. This condition would imply a constancy in both the morphological and the

physiological constitution of the system, which would be regained after disturbance. I introduce this abstract conception of the organic equilibrium in order to suggest a general point of view from which the actual phenomena of form-regulation may perhaps be considered to advantage. There is much evidence indicating that many structural correlations are controlled by electrolytic processes of the above kind.

Morphallaxis is essentially a phenomenon of form-correlation, which is exemplified by the third type of process mentioned above, in which the growth of one part may be promoted or inhibited by growth in another part more or less distant from the first. For example, in plants the development of new buds or roots is prevented so long as the main growing tip or root-system is intact; formative inhibitions of this type are extremely frequent; hence the isolation of an organ, by removing the inhibiting influence of other parts, may cause the development of roots, shoots, or other organs of a kind entirely different from itself, The whole organism may thus be reproduced in certain cases; the leaves of *Begonia* and *Bryophyllum* furnish the most striking instances of this phenomenon. In plants the normal correlation between the growth of different organs appears to depend largely on this influence. In the lower animals various regions or parts of the organism are also capable of reconstituting the whole when removed from the influence of other regions. In many axiate animals the more anterior regions thus "dominate" the more posterior regions, and a gradient of influence exists, as shown in detail by Child in his recent remarkable book.[1] The controlling influence is greatest near the dominant region and decreases with increasing distance, so that a sufficient degree of isolation for independent development may often result from simple increase in length; *e. g.*, in a planarian or a hydroid stem the portion of the animal most distant from the head end or the hydranth may form a new individual while still connected with the parent. According to Child, individuation in either asexual or sexual reproduction consists essentially in the removal of one region of the organism from the growth-inhibiting influence of other regions; the region thus physiologically isolated then

[1] "Senescence and Rejuvenescence," especially Chapter 9. See also the same author's "Individuality in Organisms."

develops on its own account. Cases of compensatory regulation,[1] *i. e.*, where the rate of regeneration is greater the greater the number of the missing parts, may be understood as due to the more complete removal of the inhibiting influence exerted normally by those parts. In other cases one region may promote or reinforce the growth or development of another region; the nervous system appears to have this relation to regeneration in a number of cases; the presence of sense-organs promotes regeneration in Cassiopæa; the intestinal tract has a similar influence in planarians.[2]

Some of the more suggestive parallels between regenerative or growth processes and the behavior of combinations of filament-forming metals have already been pointed out. The formation of precipitation-filaments from one metal is inhibited by the contact of another metal of higher solution-tension. Hence an iron wire connected with a piece of manganese, aluminium, zinc, or magnesium remains free from filaments for a considerable distance from the region of contact. If a piece of such wire be isolated by cutting, it immediately develops filaments. Similarly iron wire connected with one of these metals through a short copper wire develops no filaments; cutting the connecting wire immediately initiates their formation. These instances are sufficient for illustration of this kind of effect. The precise results obtained vary with the special nature of the conditions, and may be controlled at will by the experimenter.

The reverse class of cases where the formation of filaments is promoted, instead of inhibited, may be illustrated by a strip of zinc in contact at one end with a piece of carbon, platinum, copper or iron. Filaments are formed from all parts of the zinc surface, most rapidly near the region of contact. If then part of the zinc is severed from its connection with the other metal the development of filaments from the isolated part is at once checked; similarly with the whole strip if the platinum or copper is removed. One might compare the influence of the latter

[1] *Cf.*, *e. g.*, Zeleny's work on brittle stars and other forms, *Jour. Exper. Zoöl.*, 1904, Vol. 2, p. 1.

[2] Instances of the influence of the nervous system and sense-organs have been already cited; for the case of the intestinal tract, *cf.* Bardeen, *Amer. Journ. Physiol.*, 1901, Vol. 5, p. 1.

metal with that of the sense-organs in a regenerating portion of a medusa. The influence of the nucleus in cell-regeneration and cell-growth is another possible parallel of a more general kind.

Cases of reciprocal influence, where two or more different organs exercise mutual control upon one another's growth or development are also frequent in organisms. The development of roots and shoots from the leaves and stem of *Bryophyllum*, as recently described by Loeb,[1] furnishes very clear illustrations of this phenomenon; the growth of a bud on a piece of stem inhibits the growth of roots on an attached leaf; if the bud is removed, roots grow out; similarly roots growing from the stem inhibit the formation of shoots from a leaf. Such cases resemble the above combination where zinc in contact with a copper wire inhibits the development of filaments from iron also in contact with the wire; this influence is also reciprocal, although zinc, because of its greater solution-tension, has the predominant effect.[2] In the inorganic model the direction and intensity of the electrical current flowing between the precipitate-forming metal and the solution, and hence the rate and the character of the structural development, depend upon the special characteristics of the two intersecting circuits. The conditions in the living organism are probably similar in a general sense; and compensations and reinforcements occur, varying according to the character of the connections, the distances and other space-relations, the electrical resistances, etc. Other factors, however, including probably the flow of dissolved materials, almost certainly enter in actual cases of growth and regeneration in organisms. Nevertheless it appears probable that the most

[1] J. Loeb, *Botanical Gazette*, 1915, Vol. 60, p. 249; 1916, Vol. 62, p. 293; 1917, Vol. 63, p. 25.

[2] Loeb enunciates the rule: "If an organ *a* inhibits the regeneration or growth in an organ *b*, the organ *b* often accelerates and favors the regeneration in *a*." (*loc. cit.*, 1915, p. 276). This kind of relationship is also exemplified by the mutual influence of zinc (= *a*) and iron (= *b*) in direct contact with each other. Other well-known general facts of regeneration may be simulated by arrangements of the above kind. Thus the development of a polyp on a tubularian stem inhibits the development of polyps at other regions of the same stem, just as the outgrowth of a shoot on a plant stem inhibits growths from neighboring axils, etc. Similarly the formation of precipitation-filaments from a piece of zinc in contact with a copper wire is checked by the contact of another piece of zinc (or iron, etc.) with the same wire at not too great a distance.

general and fundamental basis of a reciprocality of this kind is electrical; morphological polarity, a closely related phenomenon, would then have a similar basis,[1] and probably also other cases of reciprocality, like reciprocal inhibition in the nervous system. It is well to recognize, however, that together with this factor others often undoubtedly enter, partly independent, partly dependent upon the electrical factor.[2]

The distances through which these inhibitory and accelerating influences are perceptible in metals vary with the conditions, the chief factors being the nature of the metals in contact and the electrical conductivity of the solution. An iron wire in contact with a metal of higher solution-tension (Al, Mg, Mn, Zn), and immersed in a 2 per cent. albumin-containing solution of K_3FeCy_6, remains free from filaments for a distance of several centimeters from the region of contact. Conversely, a strip of zinc in contact with a nobler metal (Pt, Cu, Fe) or carbon shows an accelerated formation of filaments for a similar distance. Theoretically, the current of the circuit extends through the whole of the metal and the adjoining solution; the intensity of the current between metal and solution at any point is a measure of the rate of electrolysis at that point (Faraday's law); this intensity is determined by the E.M.F. of the circuit and by the resistance of the intervening stretch of solution (that of the metal being negligible in comparison). The rate of action is thus greatest near the metallic junction, and grades off from there. A similar rule will hold for the formative or other influence of a circuit arising through local alteration in a living cell, tissue, or organism. Any local alteration of potential, due to injury, increased metabolic activity, or other condition, may thus exert an electrical influence at a distance from the site of alteration. In the case of a single cell, or other continuous element like a nerve, a current typically flows through the extracellular part of the circuit *toward* the region of alteration or physiological activity; presumably such a current produces at different points along its course the usual physiological effects of electrical currents, varying according to its intensity, local density, time-relations, etc. It is to be pre-

[1] *Cf.* Mathews, *Amer. Journ. Physiol.*, 1903, Vol. 8, p. 294.

[2] *E. g.*, the flow of a hormone-containing solution may depend on an electrical endosmose effect, etc.

sumed that in a similar manner an actively growing region has a different potential from a quiescent region, and that the circuit which includes this active region influences processes in the tissue at a distance. Mathews found the regenerating end of a Tubularia stem *negative* relatively to other regions (in the same sense in which an active or injured region of, *e. g.*, a nerve fiber is negative; *i. e.*, within the living element the positive stream flows away from the active region); and he points out the possibility that the current thus traversing the tissue may influence metabolic and other physiological processes at different regions along its course, and thus may be an important factor in the initiation and coördination of the formative or other activities concerned in regeneration.[1] With this general conception I am in full agreement. The experiments described above show in how sensitive a manner the rate of formation of precipitation structures responds to variations in the intensity and direction of the local electrical currents traversing the system; and the structural and other conditions in living systems are sufficiently like those in the artificial models to warrant the belief that electrical currents must influence in a similar manner the physiological formative processes. The closeness of many of the above parallels between the behavior of the two classes of systems confirms this belief.

It should be emphasized that the mode of transmission which prevails in processes of growth and development appears to be fundamentally of the same nature as the mode of transmission of the excitation-state in irritable and conducting tissues like muscle and nerve. I have dealt with the special features of this latter mode of transmission in a recent series of papers in the *American Journal of Physiology*.[2] In this case the bioelectric circuit between the active and the inactive regions of the irritable element appears to be responsible for the transmission of the physiological effect; the current traversing the adjoining resting

[1] *Cf.* Mathews, *loc. cit.*, p. 299. The following quotation from Pfeffer's "Physiology of Plants" is also apposite here: "Weak electric currents continually circulate in plants"; these are "partly maintained by chemical and physical agencies at work in the plant itself. It is possible that these currents may influence metabolism," etc. (English translation, Vol. 2, p. 106.)

[2] *Amer. Journ. Physiol.*, 1914, Vol. 34, p. 414; 1915, Vol. 37, p. 348; 1916, Vol. 41, p. 126.

region initiates there the same state of activity as that originally excited at the region of stimulation; the region which thus secondarily becomes active repeats the effect at regions beyond, and so the impulse spreads. There need be no decrement in this type of transmission, since an effect of the same kind and degree is automatically reduplicated at each point reached by the excitation-wave as it passes along the tissue.[1] In the transmission of the influence of a growing or regenerating or otherwise physiologically dominant region this reduplication is apparently not present, and hence a gradient of influence or "decrement" is shown. Otherwise the conditions are to be regarded as essentially the same in both types of transmission. It is possible, however, that combinations of the two may occur, giving rise to intermediate conditions.

Transmission of formative influence in development or growth, and transmission of the nervous type, are thus seen, according to the present theory, to be simply different manifestations of the same fundamental type of phenomenon, namely the transmission of an electrical influence associated with a flow of current around the bioelectric circuit. The physiological effects depend upon this current. Such a theory unifies a large number of apparently diverse phenomena. Thus the connection between growth processes and the assumed flow of current becomes intelligible if processes of electrolysis are in fact concerned in organic growth, as well as in the growth of the artificial formations described above. It also accounts for the rapidity of the nervous type of transmission in its most highly developed form, as pointed out in my earlier papers just cited. The transmission of the electrical influence through a circuit is instantaneous, whether

[1] Child assumes the existence of a decrement in the nervous type of transmission, similar to that observed in the transmission of formative influence; but this assumption seems to be unnecessary. The existence of trapped excitation-waves in rings of tissue, lasting in some cases for days—*e. g.*, in the medusa *Cassiopea* according to Mayer (*cf. Amer. Journ. Physiol.*, 1916, Vol. 39, p. 378)—proves that the impulse is renewed or recreated with undiminished intensity at each region reached by it in its course. In such a case there is obviously no decrement. It is now well established that the "all-or-none" law holds for nerve; this fact in itself would be incompatible with the existence of a decrement, since it implies that each region responds with the same degree of intensity, irrespective of the strength of the stimulus. The existence of a decrement in nerve during fatigue, anæsthesia, or asphyxia does not prove its existence under normal conditions.

the conductor be electrolytic or metallic.[1] A local change of potential in a stretch of nerve thus produces instantly an electrical change at all points of the circuit constituted by the nerve and its medium, including therefore inactive regions of the tissue at a distance from the immediate site of alteration; and if the effect at any such region be sufficiently intense, a second state of excitation is there aroused, and a similar effect is transmitted from this second region to other resting regions beyond. As a striking concrete illustration of the instantaneous transmission of electrolytic effects along a conductor to a distance from the region immediately affected, I recommend the following simple experiment. A straight piece of copper or platinum wire, *e. g.*, 20 cm. long, is immersed in a vessel containing dilute H_2SO_4; the wire remains unaltered. If then one end is touched with a piece of zinc, instantly bubbles of hydrogen start out from the surface of the wire along its entire length. The transmission of the effects of a local stimulus in such a tissue as nerve is conditioned by a process of the same type, according to the present theory; hence it is independent of the transfer of material; and the velocity with which the impulse is propagated is limited only by the degree of sensitivity of the tissue to slight electrical disturbance and by the rapidity with which the local response takes place.[2]

[1] *I. e.*, the speed is that of transmission through the ether—equal to the velocity of light, or of electricity through a metallic conductor (apart from retardation due to electrostatic influences or self-induction). An experimental investigation of the velocity of the electric current through solutions is described in the book by Monroe Hopkins, "Experimental Electrochemistry" (London, 1905). The author reaches the conclusion: "An electrolyte solution conducts the electric current as rapidly as a conductor of the first class, regardless of its composition, provided we have an equal ohmic resistance of a non-inductive type" (p. 76).

[2] In a recent article (*Amer. Journ. Physiol.*, 1917, Vol. 42, p. 469) Mayer has objected to this theory of transmission (which I have called the "local action theory") on the ground that it is "too simple." Nothing, however, but the speed of transmission of the excitation-wave, and the identity of its effects with electrical effects (stimulation, inhibition, etc.), is directly explained by the theory in question. The *entire* phenomenon of nerve conduction is indeed exceedingly complex, including metabolic and other factors of a nature as yet imperfectly known; but the detailed nature of these factors need not be regarded by this general theory, which aims merely at accounting for the possibility and rate of nervous and other forms of physiological transmission where direct transfer of material does not enter. This it does by referring the phenomenon to a more general class, regarding which our

Further light appears to be thrown upon the connection between bioelectric currents and formative processes by the above described phenomenon of rhythmical cilia-like vibratory movements of precipitation filaments. This phenomenon is frequent in newly formed filaments from iron and copper. The rhythm is typically slow, varying from one to five per second, and the movements are found only in the smaller filaments during the earliest stages of their formation, usually ceasing within a minute or two after their first appearance; at times, however, they may last for five minutes or more, especially with copper filaments. It appears probable that the phenomenon depends upon a rhythmical intermittency in the outflow of the solution contained within the tubular filament. As shown above, the metallic area at the base of the filament is an anode, the corresponding cathode being situated outside. The flow of solution through the tube is continuous so long as the extremity remains open, and is accompanied by an electric current. If, however, the opening be temporarily sealed by the deposition of precipitate, both the liquid flow and the electric current must at once cease; such a cessation of the liquid flow probably has a certain mechanical effect upon the position of the filament, while the electrical variation causes a change of surface-tension; and it seems likely that both effects combine to cause a movement, which has the effect of reopening the extremity of the filament and renewing the former condition. A rhythm is thus started which may continue for some time with often remarkable regularity. The determining condition of the phenomenon would then be the alternate disruption and reformation of a part of the tubular membrane, with corresponding opening and closing of the electrical circuit through the tube; the mechanical effects would result partly from the interruption of the liquid flow, but probably chiefly from the electrocapillary variation. Both the energy and the material for the process are furnished by the electric current through the tube.

knowledge is in many respects exact. If the transmission of the local excitation-state is due to the formation of local electrical circuits having the properties of electrical circuits in general, the difficulty of accounting for the rapid propagation of the excitation-wave along a highly irritable and rapidly responding element like a nerve-fibre at once disappears.

The comparison with ciliary movement may with advantage be pursued somewhat further. In the living structure the continuance of the vibratory movement has been shown in many instances to be dependent upon the presence of the so-called basal particle (or "blepharoplast"), which is also the center out of which the structure grows in its development; severance of the ciliary filament from its base instantly arrests the movement.[1] A connection between the conditions of contractile activity and those of growth is thus shown; both processes depend upon changes taking place in the basal granule, which represents a specially modified portion of the protoplasmic surface layer, differing in chemical composition from other regions of the cell-surface (as shown by its staining reactions), and presumably also in electrical potential. This connection becomes intelligible if we assume that (just as in the above inorganic model) the basal particle represents an area from which a supply of formative and energy-yielding material flows out along the filament to replace the material which is altered or consumed at each ciliary stroke. This flow of material is to be regarded as associated with an electrical current, which being interrupted intermittently causes intermittent polarization-effects, with resultant variations of surface-tension producing the contractions. It is clear that a constant renewal of substance is necessary to maintain the integrity of the cilium and to furnish the energy of its contractions; and the resemblance between these structures and the artificial filaments described above appears to be too close not to be based upon some fundamental identity in the conditions of both their origin and their activity. The wide distribution of these vibratile cellular outgrowths, ranging from the lowest unicellular organisms to the highest animals, as well as the readiness with which they are formed at the free surfaces of cells, indicates that some general and simple condition determines their formation, and also that the conditions of their formation and of their contractile activity are closely connected.[2]

[1] *Cf.* Peter, *Anatomischer Anzeiger*, 1899, Vol. 15, p. 271; Verworn, *Pflüger's Archiv*, 1890, Vol. 48, p. 149.

[2] The fact that bioelectric variations in other active tissues (nerve, muscle) are so frequently rhythmical in character is significant in relation to the problem of the conditions of ciliary movement. It is possible that a rhythm or intermittency

Another remarkable feature of these precipitation-tubules, already described, is that their formation is associated with a centrifugal flow of fluid along the tubule. This is not surprising when it is considered that they originate by a process of electrolysis at local electrical circuits. The passage of an electrical current through a porous diaphragm or other system of capillary tubes has long been known to involve a flow of the solution through the tubes; this is the phenomenon of fluid-transport by the current known as electrical endosmose. The possibility that this process plays a part in the transport of fluid through cells, *e. g.*, in secretion, has at times been touched upon by physiologists;[1] but in the absence of positive evidence it has been for the most part disregarded. Nevertheless it seems certain that currents of fluid must accompany the passage of electrical currents through or between cells, and also that the flow must be in general in the direction of the *positive* stream. Living protoplasm is a polyphasic system, largely fluid in its consistency, and pervaded by solid structural elements (membranes, fibrils, etc.) composed of colloidal material (protein, lipoid) which is charged negatively in contact with neutral or slightly alkaline media, such as those forming the tissue-fluids and the fluid part of protoplasm. The fluid layers in contact with such structures will accordingly be *positively* charged; and displacement of this fluid in the direction of the positive stream is inevitable if the protoplasm forms part of an electrical circuit. Protoplasm always contains salts and is a good conductor of electricity, and its enclosing membranes are readily permeable to water; hence the conditions for such a flow are present. Accordingly we should expect the passage of currents, bioelectric and other, through cells to be associated with a flow of fluid. Apart from such *a priori* considerations, there is ample evidence

in the state of electrical surface-polarization is general in living cells, and that the activity of cilia—contractile prolongations of the cell-surface—is one index of this condition. Any free cell-surface having intermittent polarization might thus give rise to cilia.

[1] *Cf.* Engelmann, *Pflüger's Archiv*, 1872, Vol. 6, p. 97; Waymouth Reid, *Philosophical Transactions*, 1900, Ser. B, Vol. 192, p. 239; Höber, *Pflüger's Archiv*, 1904, Vol. 101, p. 607. Experiments showing transport of water through tissues (muscle and nerve) by the constant current in the direction of the positive stream are described by Hermann, *Pflüger's Archiv*, 1897, Vol. 67, p. 240.

in many physiological processes of a movement of fluid through cells, or from one region to another within cells, *e. g.*, the activities of gland-cells—which are normally associated with bioelectric currents flowing in the cells toward the secreting surface[1]—and the protoplasmic flowing movements in plant-cells. Certain recent observations of Chambers[2] on the micro-dissection of sea-urchin eggs illustrate the readiness with which flowing movements may be set up in protoplasm by local injury of the cell-surface, *i. e.*, by conditions which are known in general to render the cell-surface locally negative. The application of a needle to a sea-urchin egg in a hanging drop "produces peculiar currents in the egg-substance. The currents pass directly from the pushing object in a straight line through the egg to the anterior end where they curve outward and flow back along the surface to be caught again in the flow from the pushing object." The region of contact is presumably negative relatively to other regions of the cell-surface, *i. e.*, the positive stream flows outside the cell from the unaltered to the altered part of the surface and thence through the cell; the observed flow of fluid is thus in the direction of the positive stream. The possibility that dissolved materials may be thus transported from one region of the cell to another ought especially to be noted. If such a condition is general, a flow of fluid in reference to any temporarily altered region of the cell-surface will take place as a result of electrical transport; and this flow may largely determine the supply of material for the repair or reconstitution of the altered area. The fact that in the microscopic precipitation-tubules described in this paper a flow of fluid, due apparently to electrical transport, actually plays an essential part in the structure-forming process, is highly suggestive in relation to the problem of the conditions of structure-formation in cells. There is also evidence that a centripetal flow of fluid from the polar and extra-equatorial regions of the cell-surface takes place in eggs

[1] *Cf.* Langley, Schäfer's "Textbook of Physiology," Vol. 1, p. 517. Unfortunately this observation does not in itself indicate whether the electric current is the *cause* or *result* of the movement of fluid through the gland. A flow of solution through a porous partition, however caused, gives rise to an electric current.

[2] Chambers, "Microdissection Studies," *Amer. Journ. Physiol.*, 1917, Vol. 43, p. 1; *cf.* p. 7; also *Journal of Exper. Zoöl.*, 1917.

and other cells during mitotic division, and that the astral rays are in part an expression of this flow, the central "sphere" representing an accumulation of the more fluid part of the protoplasm. Chambers brings evidence from micro-dissection favoring the view that the sphere is formed by the confluence of a system of radiating channels containing a more fluid "hyaloplasm" separated by radiating protoplasmic tracts of a solid or gel-like consistency. If the general surface of the dividing cell outside the equatorial area is electrically negative (in the usual physiological sense) relatively to this latter area, as the character of the form-change and certain other facts indicate,[1] then the positive stream in the cell-circuit must enter the surface at the polar and circumpolar areas and leave at the equatorial area. The conditions for a centripetal flow of fluid from the polar areas, due to electrical endosmose, would thus be furnished. The relation of such a flow to the general form-characters of the mitotic figure should be reconsidered in the light of these possibilities.

IV. The Possibility of Electrolysis at the Semipermeable Cell-boundaries.

Throughout the foregoing comparison between electrolytic precipitation-growths and organic growths it has been assumed that the formation of electrical circuits between different regions of the cell-surface, and associated with processes of the nature of electrolysis, is possible; and that the conditions under which such circuits arise are of the same essential nature as those determining the formation of local circuits between different parts of a metallic surface. This assumption may not appear at first sight to be consistent with our knowledge of the general conditions under which circuits are formed by chemical action, and electrolysis takes place. In the kind of process considered as typical by electrochemists, a part of the circuit always consists of a metallic conductor; this connects the two ion-forming or ion-combining surfaces (electrodes) which are in contact with the electrolyte-solution. In the living cell it is obvious that no conductor of the first class is present. In other respects, how-

[1] *Cf.* R. S. Lillie, *Journ. Exper. Zoöl.*, 1916, Vol. 21, p. 369.

ever, the cell-surface resembles the surface of a metallic electrode; local alteration—injury, stimulation, chemical change—sets up local differences of potential, and currents flow between altered and unaltered regions. The potentials thus arising may be summed, polarizability is marked, and the cell-surface is water-insoluble—*i. e.*, there is a sharply defined boundary between protoplasm and surrounding medium. The chief difference in the conditions is that the conduction of electricity both inside and outside of the cell in a bioelectric circuit takes place entirely by means of conductors of the second class, *i. e.*, by ionic movement. Both protoplasm and medium are as a rule good electrolytic conductors; and the surface of separation consists of a thin semi-permeable partition composed of chemically alterable material which is of course non-metallic in character. Can true electrical circuits, in which chemical decompositions of the nature of electrolysis take place at the interfaces, originate under conditions of his kind? This question must be considered before the above comparison can be regarded as a valid one and acceptable conclusions be based upon it.

First of all it is necessary to guard against arbitrary preconceptions of the necessary character and arrangement of the different components present in an electrical circuit depending on chemical action, such as we are familiar with in the different types of battery. In all batteries there are at least two contiguous conducting media, the one metallic, the other consisting of an electrolyte-solution; at the boundary-surface between metal and electrolyte chemical changes takes place which furnish the current; the energy of the latter is thus transformed chemical energy. In the usual diagrammatic representation of these conditions, the terminals of the one conducting medium, the metal, are regarded as immersed in the other, *i. e.*, the electrolyte; these terminals, where electricity passes between metal and solution, are the *electrodes*, while the intervening stretch of metal is usually conceived as a wire passing through the air. This last, however, is obviously an arbitrary laboratory arrangement; it facilitates consideration of the processes at either electrode to have each isolated from the other, and the connecting wire removed from the possibility of chemical change; the regions where the essen-

tial reactions occur are thus spatially distinct and the processes can be investigated separately. But evidently immersion of the conducting wire in the solution does not alter the conditions in any essential way. A piece of metal completely immersed in an electrolyte-solution, and of different composition or solution-tension at any two regions, or in contact at two areas with different solutions, is equally the seat of chemical change which is associated with the passage of an electric current between the two regions. This is the arrangement which corresponds more obviously to the conditions usually met with in nature. The case of local rusting in iron, or of the transmission of chemical influence (*e. g.*, excitation) from one region of a cell to another, exemplifies such an arrangement. It is important not to allow our conceptions of natural processes to be limited by the peculiarities of laboratory devices; these are always more or less arbitrary, and designed not only for convenience and reduplication at will, but with the special purpose of isolating the phenomena and making them as sharply defined as possible. In unmodified nature such conditions are rarely found; hence such resemblances as that between local chemical action in metals and the physiological effects following local alteration in living cells are not easily recognized.

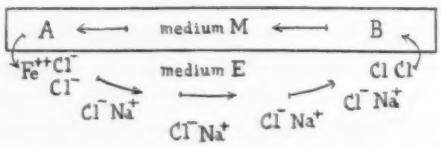


FIG. 1. In this diagram medium *M* represents the metallic part of the circuit, *e. g.*, a platinum wire, medium *E* the adjoining electrolyte solution, *e. g.*, NaCl. Ferrous chloride is in contact with the wire at one end (*A*), chlorine at the other (*B*). The arrows show the direction of the electrical current (positive stream) in the circuit.

A simple type of oxidation and reduction cell seems best adapted to illustrate the essential nature of the phenomena under consideration, especially since the energy of vital processes, including that of the bioelectric currents, is usually derived from oxidations. Probably the simplest case is that of an easily oxidizable salt, *e. g.*, ferrous chloride, in contact with a chemically

indifferent conductor, *e. g.*, carbon or platinum, which is immersed in a solution of an electrolyte, *e. g.*, NaCl. The ferrous salt may be in contact with only one region of the platinum wire, *e. g.*, one of its extremities; if now an oxidizer (*e. g.*, chlorine, bromine, nitric acid, etc.) be brought in contact with the other end of the wire, the ferrous salt is at once oxidized to ferric. The subjoined diagram (Fig. 1) will illustrate, in which chlorine is regarded as the oxidizer. The rate of the process depends upon the concentrations of the $FeCl_2$ and Cl_2 at the electrode regions, the area of the electrodes, and the electrical conductivity of the circuit. Each atom of chlorine reaching the wire at region B causes instant oxidation of a molecule of $FeCl_2$ to $FeCl_3$; *i. e.*, each ferrous ion (Fe^{++}) becomes a ferric ion (Fe^{+++}) by receipt of a positive charge from the wire; this charge comes from the chlorine atom which releases it to the wire, becoming at the same time a chlorine ion (Cl^-). The number of chlorine ions in solution thus necessarily increases at the same rate as the number of ferric ions, and the only evident chemical changes are those occurring at the contact of the oxidizing and reducing substances with the wire. The current (positive stream) flows in the solution from the iron salt to the chlorine, and a certain process of rearrangement or change of ionic partners may be conceived as taking place throughout the whole intervening stretch of electrolyte-solution.[1]

The above account will indicate sufficiently the general character of the physical and chemical changes taking place in any oxidation and reduction cell. At the anodic area Fe^{++} ions are oxidized to Fe^{+++} ions; at the cathodic area electrically neutral chlorine atoms are reduced to Cl^- ions. The wire serves simply to convey electricity from the one region to the other. Similarly

[1] It is impossible to give a full exposition in this place, and the reader is referred to the textbooks of electrochemistry (Arrhenius, LeBlanc, Lüpke, etc.) for a more detailed treatment. The electron theory includes all of the above phenomena as special cases. From the standpoint of this theory it is more correct to regard each Fe^{++} ion as yielding an electron (negative charge) to the wire at region *A*; this displaces a corresponding electron at *B*; this electron is taken up by the Cl atom which thus becomes a Cl^- ion. The flow of electrons constitutes the current in the wire; at the electrodes there is equal and simultaneous loss and gain of electrons by the substances (ions or other dissolved substances) at anode and cathode respectively.

any oxidizer which is capable of being reduced by $FeCl_2$ may be substituted for chlorine; and any substance which is capable of being oxidized by the oxidizer may be substituted for the ferrous salt.[1] The above substances serve merely to furnish the simplest possible illustration. Oxidation at the anode, reduction at the cathode, with accompanying flow of current between the two regions, form the constant features of the phenomenon; obviously the energy of the current is derived from the energy of the oxidation. It will thus be readily understood that the chemical changes at the electrodes may in some cases be much more complex than those cited above for illustration; and that under certain conditions they may result in decompositions and syntheses of a far-reaching character.

In the above diagram one half of the circuit, designated as medium *M*, is regarded as consisting of a metallic conductor which itself undergoes no chemical change; the other half, medium *E*, is the electrolyte solution. Obviously a metallic conductor which is not chemically indifferent but is capable of combining chemically with the ions of the electrolyte-solution will produce similar electrical effects. A metal of low solution-tension like zinc or iron will contribute cations to the solution at the anode; these will form chemical combinations according to the nature of the substances present in the solution. Or the reducing influence at the cathode will affect all reducible substances there present; if, *e. g.*, the cathode is composed of a conducting oxide like copper oxide, as in the Edison cell, this is reduced to the metallic state. Evidently the more complex the material of anode and cathode, and the more complex the contiguous solution, the more numerous the chemical transformations that may occur. In all cases, however, Faraday's law holds, and the energy freed in the resulting process is strictly dependent upon the difference in the chemical potential of the substances undergoing transformation at the two regions.

The case does not appear to be altered in essential principle when medium *M* is considered to be not a metal, but a second

[1] See the text-books of electrochemistry for examples of the various possible combinations. An oxidizable organic substance, *e. g.*, pyrogallol, α-naphthol, sugar, may be the substance at the anode; a peroxide the oxidizer at the cathode. These examples are adduced to suggest biological possibilities.

electrolyte-solution, separated from medium *E* by a thin partition which prevents diffusion (*i. e.*, is semi-permeable) and can undergo the chemical changes necessary for the generation of an electric current, *e. g.*, oxidation at one region simultaneously with reduction at another. Under these conditions medium *M* may be permanently of a different composition from medium *E*, both are electrical conductors of the electrolytic kind, and local chemical changes resulting in ionization or deionization within the substance of the partition-film will result in corresponding electrical effects which may be transmitted and produce chemical effects at a distance just as in the case considered above. This appears to be the type of arrangement exemplified in the living cell.

To understand how such currents may be formed without a metallic conductor it is necessary to consider more closely the nature of the part played by this conductor in the usual type of circuit. In the above example the platinum wire serves simply as a means for transmitting electricity, a positive charge passing to (or a negative away from) the metal from each Cl atom and from the metal to each Fe^{++} ion. Electroneutrality is thus preserved on either side of the interface; the only region where chemical change occurs is where electricity is thus added to or abstracted from the substances in contact with the electrodes. This last is the essential condition of all electrolyses; obviously the precise character of the chemical transformations in any special instance depends on the nature of the substances present at the electrodes. Extensive molecular rearrangements may take place under some conditions; thus the anodic oxidation may result in syntheses as well as in decompositions; similarly with the reductions at the cathode. In the interior of the wire no permanent change occurs, since at each region electrons are simultaneously entering from the one side and leaving from the other in equal quantity, leaving the local condition unaltered. In the interior of the solution diffusion-changes may occur; but apparently there are no interionic or intermolecular transfers of electrons, since the quantity of electricity passing through the circuit is strictly proportional to the number of ions reaching the electrodes and charging or discharging there (Faraday's

law).[1] The source of the current is thus the chemical changes taking place at the electrodes; in these changes electricity is added to the wire at one region and an equal quantity is simultaneously abstracted at another. Precisely the same chemical change takes place and the same quantity of energy is freed when the chlorine is brought directly into contact with the solution of ferrous salt; here the transfer of negative electricity from the ferrous ions to the chlorine atoms takes place without the intermediary of a metallic conductor. These considerations show that the metallic conductor, by acting as a source of electrons, merely *enables* the change of valence to take place without the direct contact of the chlorine and the ferrous salt. This effect follows because the affinity of the chlorine atom for a negative charge (*i. e.*, electron) is greater than that of the ferrous ion; the chlorine atom is thus able to abstract a negative charge from the metal and impel the ferrous ion to yield up a similar charge to it,—*i. e.*, to assume an additional positive charge and become a ferric ion. The latter then interacts chemically with whatever other substances are available.[2]

In the oxidation and reduction circuit considered above, the current on the one side of the interface, *i. e.*, in the metal, is conducted by a simple movement of electrons without chemical change; on the other side, *i. e.*, in the solution, it is conducted by the diffusion of ions and the charging or discharging of ions at the electrode-areas, with consequent local chemical change. Let us now suppose that instead of the combination of a metallic conductor and an electrolyte-solution, we have *two* electrolyte solutions separated by a thin semi-permeable partition the substance of which can combine chemically with the ions of one of the solutions. Such an arrangement corresponds to the case

[1] Were this otherwise, electricity could flow through the solution by shifting of electrons from ion to ion (or molecule to molecule, etc.) without the transport of the ion as a whole. This, however, does not seem to occur (unless the current-intensity is very high). It is only when the ion interacts chemically with the substances at the electrode, or with the electrode itself, that any flow of current can take place. This is why electrolysis is always associated with the passage of a current through a solution. The metallic state appears to be characterized by a relatively high concentration and free mobility of electrons.

[2] The presence of potassium thiocyanate in the solution at the anode is a convenient way of demonstrating the production of ferric ions. The ferrous salt should be as free as possible from ferric admixture.

of the living cell immersed in its normal medium. The two solutions are to be conceived as differing in their electrolyte-content, so that a potential-difference exists across the membrane. If now we consider a circuit as arising between different regions of the surface in consequence of some local chemical change in the substance of the partition, such as an oxidation, it is clear that the conditions of the flow of current in both solutions must be in principle similar to those just considered; *i. e.*, the mode of current-transport accompanied by chemical change which prevails on one side of the interface in the example cited, *i. e.*, in medium *E*, may equally well prevail on *both* sides. The essential conditions are that the chemical changes taking place at the interface must be of such a kind as to furnish free energy (for the flow of current), and that the necessary electro-neutrality of either solution is preserved intact. It is not difficult to imagine the general features of such an arrangement, although as regards the special nature of the conditions existing in living cells (the substances involved, the nature of the reactions, etc.) little definite can be said at present. In brief, then, the present conception regards the current in the interior of either solution as transported by the movement of ions in the usual manner; but in its transport across the boundary-partition chemical reactions are concerned; *i. e.*, in this part of the circuit the essential change consists in transfers of electrons between interacting molecules or ions with corresponding chemical reactions which furnish the energy of the current. Such processes would be conditioned in the same essential manner as the processes at metallic electrodes, *i. e.*, would be of the nature of electrolysis, since they would depend upon the flow of the current through the circuit. Hence they would be influenced by electric currents from outside sources, besides giving rise to currents when they occur of their own accord under normal conditions. It seems highly probable that the characteristic electrical properties of living cells depend upon arrangements of essentially this kind; the fundamental general fact that cell-activities both give rise to electrical currents, and are themselves profoundly influenced by currents of outside origin, is most readily understood on this hypothesis.

The special nature of the conditions in living cells may now

be briefly considered, although for the present little more can be done than to indicate the probable class to which the phenomena belong; the elucidation of details is a task for future research. In general the film separating the protoplasm from the external medium is thin and semipermeable, and the solutions on either side of this partition are of different composition. Hence the conditions for a potential-difference between protoplasm and medium exist; and such a state of electrical surface-polarization is always found. This polarization varies characteristically with the nature and the physiological activity of the cell; and circuits are thus continually arising in active tissues (like muscle, nerve, etc.) between different regions of the cell-surface. The conditions under which the normal bioelectric circuits originate seem most closely analogous to those prevailing in such an arrangement as the "oxygen-hydrogen cell" of electrochemistry, in which two platinum electrodes, charged respectively with oxygen and hydrogen, are immersed in an electrolyte-solution. In this case the oxygen electrode forms the cathode, the hydrogen, the anode; the oxygen gives up positive charges to the platinum when the circuit is closed, while the hydrogen receives such charges from the metal; the oxygen thus forms oxygen- or hydroxyl-ions, the hydrogen forms hydrogen-ions; and the union of these to form water furnishes the energy of the current. Let us suppose—to make the case concrete—that the external solution bathing the living cell contains oxygen, and that the surface-film consists of oxidizable material. In the resting condition of the cell there is a certain tendency for the oxidation to take place (or oxidation-tension), *i. e.*, for the oxygen to impart positive charges to the oxidizable partition, becoming itself ionized; but this tendency is held in check—just as in the oxygen-electrode when the circuit is open—because such a transfer would leave unbalanced negative charges behind in the medium. Hence an equilibrium exists, similar to that at the surface of any battery-plate with open circuit. But any change of condition by which negative charges may be set free in the protoplasm at any region will disturb this equilibrium and allow the combination to take place. The oxygen then imparts its positive charges to the oxidizable material of the partition; this

on becoming positive may then combine with the negative ions present—which may be oxygen or hydroxyl or other available anions, according to concentrations, affinities, etc.—and the material assumes a higher state of oxidation; other transformations, including syntheses, may follow from this change. Such a process implies that a positive electrical stream enters the cell-surface from the medium at the site of oxidation, and leaves the cell at other regions; at the latter regions presumably cations are given off from the cell-surface (which appears to act as a reversible electrode relatively to cations). A circuit thus flows through cell and medium, the positive stream flowing in the external medium toward the region of oxidation and away from the resting or chemically inactive regions. In other words, the local combination of oxygen with the cell-surface at any region involves the entrance of positive charges at that region, in a manner analogous to the entrance of positive charges at the cathode in the oxygen-hydrogen cell. So far as the physiological evidence extends, the region of highest oxidation-rate in cells does appear always to be negative (in the physiological sense) toward regions of lower oxidation-rate. This is indicated by the general fact that stimulated or active regions are negative relatively to inactive regions, oxygen being typically required for such activity; actively growing regions appear also to be negative relatively to less active or non-growing regions; this is seen in the regeneration of hydroid stems and of the tails of fishes, and in the growth of seedlings,[1] and is probably general, although at present the observations are insufficient to establish this generalization empirically. In such cases the negative regions—*i. e.*, the regions where the positive stream enters the cells from outside—are the regions of most active constructive metabolism; and since oxygen is required for growth and regeneration, this fact may be regarded as an expression of the general connection between oxidation-processes and syntheses. It should be added that changes in the local physical or chemical

[1] *Cf.* Mathews, *loc. cit.* For the case of seedlings *cf.* Müller-Hettlingen, *Pflüger's Archiv*, 1883, Vol. 31, p. 193. Hermann first observed that the growing root-tip in seedlings was strongly negative to other regions (*Pflüger's Archiv*, 1882, Vol. 27, p. 288), and Müller-Hettlingen followed the subject further at Hermann's suggestion.

condition of the surface-film, induced (*e. g.*) by external agents, may also produce bioelectric circuits accompanied by oxidation processes; thus a region of increased permeability resulting from stimulation may be the condition disturbing the equilibrium; or a local metabolic change may take place in the surface film resulting in abstraction of cations from the cell-interior by a process of reduction; in a circuit like that imagined above such a change would result in oxidation at other regions of the cell-surface. The conditions under which local oxidations occur and bioelectric circuits originate undoubtedly vary widely in different cells, and probably also in the same cell at different times.

It thus seems clear that electrical circuits may originate through chemical reactions which take place at the boundary between solutions of different composition separated from each other by semi-permeable partitions having the properties of the plasma-membranes of living cells. There is no theoretical necessity for a conductor of the first class. Local transfers of electricity to and from the protoplasm may occur simultaneously at two different regions of the cell-surface as the result of chemical interaction between the substance of the surface-film and ions or ion-yielding substances present in the protoplasm or in the medium; circuits between these regions thus arise. The possibility of this general condition must be admitted if we accept the general electrochemical theory that the metallic conductor in (*e. g.*) an oxidation-reduction cell enables the reaction to take place simply by serving as medium for the transmission of electrical charges. The phenomenon of chemical action at a distance appears indeed to be a clear demonstration that chemical action depends upon the transfer of such charges. The production of the bioelectric circuits becomes intelligible if we assume that the charges conditioning the reactions at the electromotor surfaces may be derived from oxidizing or reducing substances present in the protoplasm, or surroundings, just as readily as from a metallic conductor.

This general conception is evidently related to the conception of ionic exchange in such a phenomenon as absorption.[1] Local

[1] See Höber's "Physikalische Chemie der Zelle," pp. 238 seq.

circuits should be equally possible in such cases, and these may be associated under certain conditions with chemical change. The action of strong electrolytes upon poorly conducting material (*e. g.*, wood, rubber, leather, etc.) is probably accompanied by electrolytic action of this or similar kind. The distinction between ordinary chemical action and chemical action due to electrolysis is in fact rendered arbitrary by the facts of chemical action at a distance. Wherever electrical transfer is possible under conditions not resulting in the development of a counter E.M.F. or other polarizing effect which arrests the current, the chemical influence may be transmitted to a distance. This explains why circuits are necessary to this process. Of course the rate of any reaction thus conditioned varies inversely with the electrical resistance of the circuit which determines it; and it is only when the resistance is relatively low—as in circuits consisting of a metallic conductor combined with an electrolyte-solution, or in circuits of two electrolyte-solutions separated by a polarizable semi-permeable partition—that such transmission can take place through considerable distances.

A difficulty may be felt in accounting for the conduction of electricity across the membrane in living cells. This is normally *semi-permeable, i. e.*, impermeable to ions and other diffusing substances; the continued existence of the cell depends in fact upon this condition. The assumption of a selective permeability to cations (or reversibility to cations) may remove the difficulty of explaining how the current can be conducted across the resting regions of the cell-surface. At the active regions conduction may take place by chemical reaction accompanied by electron-transfer, as already suggested. It seems possible, however, that at times the positive stream may pass from the cell-interior to the external medium without such chemical change; thus the addition of cations to the membrane from within, following stimulation at some other region, might disturb the polarization-equilibrium and detach corresponding cations from the outer surface; at such regions the current would flow from within outward without change in the membrane, as observed at the resting regions of the cell. In such a case the membrane would behave like a non-polarizable electrode. To what degree and

under what circumstances this possibility is realized is difficult to say. Certainly in most of the instances hitherto investigated (muscle, nerve) the passage of the constant current through the cell produces marked polarization-effects (*i. e.*, sets up immediately a strong counter E.M.F.). Nevertheless the above possibility should be considered.

V. General Conclusions.

The foregoing discussion raises the question whether in organic growth the essential structural condition is not the presence of semi-permeable and hence electrically polarized partitions separating the living substance from its medium, and at which processes of electrolysis may take place. If this is so, the prevalence of the cellular type of organization would be largely accounted for. Organic growth (as well as normal maintenance) would then involve the deposition in the continuous surface-layer of materials which preserve the character of that layer, *i. e.*, its continuity, semi-permeability, and specific physico-chemical composition. It is clear that any layer having such properties would have to form a *closed surface;* otherwise the difference between the inner and outer solutions could not persist; hence a "cell"—mass of protoplasm enclosed by a membrane—would be the living unit. A tendency to a continual increase of this surface-layer in an appropriate nutrient medium would then constitute the primary feature of the growth-process. Evidently the continued growth of such a system would involve a decrease in the ratio of surface to volume: and the need of preserving this ratio above a certain minimum may be the condition that has led to the association of cell-division with growth, as Herbert Spencer suggested long ago. According to this conception, "living" material would be essentially material at the boundary-surface or under the direct influence of the boundary-surface; definite limits would thus be imposed upon the dimensions of the individual living units. In general we find that protoplasm is partitioned freely; *i. e.*, the ratio of surface-protoplasm (membrane-protoplasm) to the total mass is usually large. The exceptions (yolk-laden eggs, muscle cells, certain plant-cells) may be explained on special grounds.

There is no doubt that the distinctive vital properties of self-maintenance, growth, and reproduction are closely interconnected; it would probably be more correct to say that they are manifestations of the same fundamental physiological activity under somewhat different conditions. The essential feature of this activity is constructive metabolism of a specific kind. The mechanism determining this process of construction must be intimately connected with the mechanism determining the response to stimulation, since stimulation, which effects a breakdown of the living substance, must itself form the condition of the restitution of the broken-down material, if the system is to continue to exist. This was clearly recognized by Claude Bernard.[1] Some kind of self-regulating cycle, in which a destructive process calls forth automatically or inevitably a counter-process of repair, would thus seem to be the necessary foundation of any form of vitality. An analogy to this condition is seen in the chemical processes of any electrolytic circuit; just as oxidation at the one electrode involves the inverse process at the other electrode, so decomposition or structural breakdown at one region of the cell-surface may, by giving rise to a local bioelectric circuit, directly determine a reparative synthesis either at the same or at an adjoining region.[2] Some general physico-chemical condition of a simple kind must be common to *all* of the various types of living system, since they are all *living*. The universality of the cellular type of organization seems—to my mind at least—to indicate that the essential structural condition in living matter is the presence of polarizable partitions at which processes of electrolysis, including both syntheses and decompositions, are to be conceived as taking place under the influence of local electrical circuits. The self-maintaining character of the living system, its responsiveness to outside influence (especially of an electrical kind), and the transmission of chemical influence to a distance, become to a considerable degree intelligible on such an hypothesis. These considerations may appear too speculative to many; but further advance in physiological analysis seems to demand that the fundamental vital processes should at least be

[1] *Cf. e. g.*, "Leçons sur les phénomènes de la vie," Vol. 1, p. 127.

[2] For further discussion of this possibility *cf.* my recent paper, *Amer. Journ. Physiol.*, 1917, Vol. 43, pp. 56, 57.

assigned to their proper physico-chemical classes. The similarities between the phenomena described in this paper and many of the most characteristic peculiarities of the growth-process in organisms seem to me to be too detailed not to signify an identity in some essential underlying condition. The need of a physico-chemical analysis of the growth-process is clear; growth is unquestionably the most fundamental of vital activities, and any adequate theory of growth would be equivalent to a theory of the vital process itself.

THE CONCRESCENCE OF FOLLICLES IN THE HYPOTYPICAL OVARY.[1]

LEO LOEB.

In a preceding paper we have shown that lack of sufficient nourishment is the cause of a hypotypical condition of the ovaries. In one guinea pig we found in addition to the hypotypical condition another peculiarity of the ovaries, which is of considerable interest and which deserves a special description. In connection with the facts reported in the preceding paper it leads to some interesting conclusions as to the effect of underfeeding on the development of the ovarian stroma, and on the development of what in the guinea pig in certain respects corresponds to the interstitial gland of the rabbit and it may throw light on the origin of follicles containing more than one egg.

Female guinea pig No. 383 was obtained from Iowa. On January 12, 1917, it weighed 475 grams; on that date both lobes of the thyroid of this animal were removed. One thyroid lobe from another guinea pig, obtained from a different breeder and weighing 380 grams, was transplanted into the subcutaneous tissue of guinea pig No. 383. The transplanted thyroid was removed for microscopic study seventeen days later, January 29. At that time the guinea pig weighed 322 grams; it had therefore lost 32 per cent. of its original weight in a period of seventeen days. The transplanted thyroid was on the whole in a very good condition; we shall refer to this aspect of our findings in another connection. Here we are especially concerned with the condition of the sexual organs. The uterus shows low or medium cylindrical epithelium of the surface and glands with a very small number of mitoses in the surface epithelium. The mucosa is thin and fibrillar but very hyperemic. In the lumen of the uterus there are some erythrocytes. The ovaries are markedly hypotypical. They contain several atretic yellow bodies, the rem-

[1] From the Department of Comparative Pathology, Washington University, Medical School, St. Louis.

nants of former corpora lutea. There are in the cortex a number of primordial follicles developing into small Graafian follicles and into follicles of small to medium size. But before they have hardly reached the latter size, a slow solution of the granulosa sets in and very soon the cavity of the follicles is lined by the theca interna.

The further course of the atresia of the follicles is, however, changed; the connective tissue does not as usual grow into the follicular cavity; instead the atretic follicles present themselves as small cysts. The ordinary follicles in various stages of connective tissue atresia are therefore lacking. There is, however, present a very large number of follicles in the last stage of atresia, a cavity with remnants of the zona pellucida, or merely a small cavity surrounded by a thick layer of the shrunken theca interna cells. By far the greater part of the ovaries consists of such follicles in the last stage of atresia; the proportion of such follicles is much greater in this animal than in normal ovaries which are in the period following the first week of ovulation and even larger than in the other hypotypical ovaries which we observed.

There are two further peculiarities noticeable in these ovaries, viz., (1) a relative decrease in the amount of fibrous tissue which surrounds the various follicles, and (2) the presence of numerous follicles with multiple eggs. While we observe around a number of growing or atretic follicles a fibrous membrane, the latter is always thin and it is absent around other follicles. Not rarely we see two adjoining follicles of small to medium size merely separated by several rows of relatively large theca interna cells; or we see small follicles close together, and not separated by fibrous tissue; at other places the theca internae of two quite atretic follicles press on each other, the theca cells of the one follicle surrounding directly the capillaries of the theca interna of the adjoining follicle. In other cases two or three shrunken egg cavities are enclosed in one mass of small theca interna cells.

If we compare the amount of fibrous tissue separating the follicles in this animal and in other animals with either normal or hypotypical ovaries, we find in the ovaries of other guinea pigs a larger amount of fibrous stroma separating the various follicles. This decrease in fibrous tissue made it possible to

differentiate microscopically the ovaries of this animal from those of other guinea pigs. There occurs in these ovaries a considerable number of follicles with two, three or even more eggs. We see in the most peripheral zone of the ovary two well preserved eggs of about the same size, but one slightly pressing upon the other and both surrounded by a single layer of granulosa cells. Somewhat further away from the tunica albuginea we see small follicles with two eggs of the same size, separated only imperfectly by a few flat granulosa cells. At other places we see follicles of small to medium size with a cavity. At the end of the cavity directed toward the center of the ovary there is a relatively large, well-preserved egg, surrounded by several rows of granulosa cells. At the side of the follicle directed toward the periphery of the ovary there is a very small, but well-preserved egg, in an earlier stage of development, surrounded by only one layer of granulosa cells. Here we have a combination of a primordial and of a small Graafian follicle. In other cases we see a small egg of a primordial follicle, pushing its way through the granulosa of the somewhat larger follicle and just reaching the cavity with the outer pole; the most frequent orientation of the two eggs is as follows: The larger one, surrounded by several rows of granulosa cells, is placed at the central pole of the cavity and the smaller one more toward the peripheral pole. But occasionally the order may be reversed, or the small egg may be at the lateral aspect of the follicle. In one case we found the egg of the primordial follicle in the suspensory ligament of the granulosa of the larger follicle. In still other cases we find a combination of a small egg at an early stage of its development, surrounded by one row of granulosa cells, the whole corresponding to a primordial follicle in the cavity of a small to medium follicle in which the granulosa and egg have already undergone degenerative changes. The degenerating ovum may still be surrounded by granulosa cells or the granulosa cells may have been dissolved. In other cases we find in a follicle of small to medium size two well developed eggs, both surrounded by several layers of granulosa cells. Similar combinations we find in follicles with three eggs. There may be combined a degenerated egg, a relatively large, preserved egg surrounded by several layers of granu-

losa, and an egg of a primordial follicle enveloped by one layer of granulosa. In a follicle in which we found a still larger number of eggs, the latter were well preserved and rather small, and situated in different parts of the granulosa. Of interest is also an observation which showed that a disintegrating egg may enter into direct contact with the theca interna of a nearby follicle of small to medium size.

These are the principal combinations of double or multiple eggs we observed in the ovaries of this animal, and both ovaries behaved in a similar manner. How shall we interpret this condition? The large number of follicles with two or more eggs which we found in both ovaries indicates clearly that we have to deal with a general condition of the organism affecting both ovaries equally and not with a local ovarian change. We may conclude that bioval or plurioval follicles may originate in either of the two following ways: (1) The young eggs in the tunica albuginea of the ovary remain united; connective tissue fails to grow between two or more eggs and to separate from each other the different eggs with the surrounding granulosa cells. The pictures of primordial follicles containing two eggs speak in favor of this interpretation. (2) But other follicles containing more than one egg must originate in a somewhat different manner. Very small follicles, especially primordial follicles, push their way into larger follicles, perhaps even into such follicles which are already in the process of degeneration. And this is probably by far the more frequent mode of origin. We find different stages in the junction of two follicles of different sizes, and in different stages of development. We can follow the pushing in of the smaller into the larger follicles. The fact that the thickness of the granulosa surrounding the different eggs in a follicle is in accordance with the character of the egg is only compatible with the view that two formerly distinct follicles effected a secondary union. In favor of this interpretation speaks also the observation that in the majority of cases the more undeveloped egg is situated toward the outer pole of the larger follicle. This is in accordance with the fact that the smaller a follicle is, the more peripheral is its situation. Occasionally, however, a primordial follicle may be pushed deeper into the cortical tissue

before it enters the wall of the larger follicle. In this case we might expect to find the smaller egg in the central part of the follicle and the larger one more toward the periphery. Whether also two small to medium follicles of similar size can unite to form one follicle with two eggs, we are unable to determine with certainty.

Essentially both modes of origin are in all probability based on the same condition, both being due to the same cause, namely, the relative inactivity of the connective tissue in these ovaries. We saw that in this animal the atresia of the follicles does not take its normal course, but that after disintegration of the follicles the connective tissue fails to grow into the cavity and that thus small follicular cysts syrrounded by theca interna are formed. We saw furthermore that the fibrous bands separating the various follicles are not so well developed as in the ovaries of other guinea pigs. It is therefore very probable that this deficiency in the connective tissue is at the bottom of this condition. The relative inactivity of the connective tissue, its failure to proliferate and to produce fibrous tissue, accounts for the imperfect atresia of the follicles and the imperfect separation of the follicles, and it is this inactivity of the connective tissue which is responsible for the occurrence of the follicles containing multiple eggs. In the first place, the connective tissue in some cases fails to grow properly between the very young eggs and their granulosa and to separate the two follicles; thus the first mode in the production of follicles with several eggs is brought about. It is due to a pathological persistence of an earlier stage in the development of follicles. And secondly the lack of development of strong fibrous tissue around a number of follicles, together with the pressure existing in the ovary as the result of the growth of young follicles, is responsible for the pushing of small follicles into larger ones. Thus there is no essential difference in the mode of origin of bioval or plurioval follicles at various stages of development.

The next question concerns the cause of this relative inactivity of the connective tissue. The study of the hypotypical follicles, which we reported in the preceding paper, throws light on this problem. We mentioned there that in cases in which the hypo-

typical condition was pronounced, there was noticeable a tendency to delay in the ingrowth of connective tissue into the follicular cavity; thus small cysts resulted. We interpreted this relative inactivity as due to the underfeeding which at last affects also the connective tissue. This condition represents a further stage in the chain of changes produced through underfeeding. This chain is as follows: (1) The maturation of follicles is suspended. (2) Abortion takes place in pregnant guinea pigs. (3) The granulosa cells of developing follicles are injured and are prematurely dissolved, but the relative strength of new formation on the one hand, and of destruction of granulosa cells on the other hand, shows such a balance in favor of new production that the development of follicles progresses so far that medium follicles are formed. (4) This balance becomes more unfavorable and only small and small to medium follicles develop; after this stage has been reached and the granulosa has been dissolved connective tissue grows in and fills the cavity and leads to a shrinking of the follicle. (5) In the last stage even the connective tissue becomes inactive. The ingrowth of connective tissue into the cavity does not take place. And the same process leads also in all probablity to an under-development of the fibrous bands separating the various follicles. Thus the separation of follicles becomes interfered with and the multiplicity of eggs in a follicle results.

It is, of course, possible that there are at work additional factors which favor such a process. It might be that in certain individuals the tendency of connective tissue to be inactive is greater than in others. It might also be that the number of the young follicles in early stages of development is greater in this animal and that thus a crowding of young follicles results. However, if such a difference exists between this and other ovaries, it is not very marked. Multiplicity of eggs in the ovarian follicles has been observed by us in several other ovaries of guinea pigs. At the time however when our previous observations were made we had not yet gained an undestanding of the connection between this change and the abnormal conditions which we found in this case. Ovarian follicles with several eggs occur also in other species, especially also in man. It would be

of interest to inquire whether in all cases the causes are the same. or whether different conditions may lead to the same result; In such an inquiry we have to keep in mind the possibility that a hypotypical condition of the ovaries might be a transitory state; that after having led to the production of follicles with multiple eggs, new follicles may again begin to develop normally, so that in the end nothing indicates that at one time such ovaries had been hypotypical.

This concrescence of follicles is somewhat analogous to a condition we find in other glands. In the thyroid of the guinea pig, as well as in the mammary gland of the mouse, we found occasionally structures which could only be explained as due to a concrescence of neighboring acini; due to the disappearance of the walls separating adjoining acini. We have therefore to deal with a phenomenon of a more general character, which under certain conditions may perhaps occur in all the glands.

There was in the ovaries of this guinea pig an additional feature which deserves special mention, viz., the very marked development of what corresponds to the interstitial gland in the rabbit. As we stated above, by far the greater part of this ovary consists of follicles in the last stage of atresia, follicles in which the shrunken cells of a well-developed theca interna surround one or several small cavities, the remnants of what were formerly follicular cavities. While we find in all hypotypical ovaries a relative preponderance of this kind of follicle, in the ovaries of this guinea pig this feature was more prominent than in the ovaries of the other animals. This is due to the following factors: (1) At the time of the thyroidectomy, the ovaries were in all probability relatively large, the animal weighing at that time 475 grams; thus space was available for the expansion of atretic follicles. (2) The follicles ceased to develop to medium or large size. Thus the pressure exerted by the larger follicles was eliminated, and a chance was given the atretic follicles to occupy the space otherwise occupied by large follicles. The slight development of fibrous bands around the follicles must have a similar effect diminishing pressure exerted on atretic follicles. We may therefore conclude that the structure analogous to the interstitial gland is prominent whenever the intra-

ovarian pressure is relatively slight. Under these circumstances the last stage of follicular atresia is preserved throughout a relatively long period of time. A similar condition we find in the ovaries of the guinea pig within the first week after ovulation, when the larger follicles have all become atretic.

Conversely we may conclude that the intraovarian pressure hastens the disappearance of the theca interna and of the other vestiges of atretic follicles. The pressure of the growing structures is stronger than the pressure of the resting and disappearing structures, and thus the life and growth in one part of the ovary hastens the disappearance of degenerating structures elsewhere. Not only is the disappearance of the shrunken theca internæ thus hastened, but also that of the fibrous tissue structures in the ovary. In our case it is uncertain whether the connective tissue had formerly grown into the follicles which are now in the last stage of atresia, this connective tissue having been absorbed subsequently, or whether in this animal the last stage of connective tissue atresia was reached directly from the cystic condition through absorption of the fluid in the follicular cavity, without a preceding ingrowth of fibrous tissue. In case the latter alternative should hold good the lack of ingrowth of connective tissue into atretic follicles would have been present for some time previous to the examination of the ovaries. In some atretic follicles we notice however some vestiges of fibrous tissue around the central cavity and it is therefore probable that in these cases the connective tissue had previously grown into the cavity after the solution of the granulosa. We also must consider the possibility that diminished intraovarian pressure may in itself influence the activity of the connective tissue.

Summary.

We describe the ovaries of a guinea pig in which the experimentally produced hypotypical condition was more advanced than in any of the other animals which we observed. In this case the underfeeding not only affected the activity of the granulosa, but also of the connective tissue. In consequence of a lowered activity of the connective tissue a concrescence of follicles takes place in numerous cases in both ovaries. A lowering of

intraovarian pressure in such ovaries leads to a relative preponderance of what corresponds to the interstitial gland in the ovaries of certain other species. The concrescence of ovarian follicles is analogous to the union of neighboring acini in the mammary gland and thyroid. It is a phenomenon which occurs probably in all or the majority of glandular structures.

A COMPARISON OF MITOCHONDRIA IN PLANT AND ANIMAL CELLS.[1]

N. H. COWDRY,

ANATOMICAL LABORATORY, JOHNS HOPKINS UNIVERSITY.

The recent discovery, through the elaboration of new methods of technique, of the wide distribution of mitochondria has attracted the attention of many investigators in widely separate fields. Their characteristic form, strongly suggestive of bacteria, is now quite familiar. The technique was devised by anatomists and was applied by them to plant cells. I refer particularly to Meves' ('04, p. 284) work on *Nymphæa*. But, as might have been expected, these structures did not altogether escape the careful scrutiny of the older botanists, despite their imperfect methods of technique; for Zimmermann ('93, p. 215) certainly observed and described mitochondria in the living hair cells of *Momordica elaterium* and in the meristem and root tip of *Vicia faba*. Nevertheless botanists in this country have been slow to study mitochondria notwithstanding the fact that in properly made preparations they constitute a cell organ as conspicuous as the nucleus.

Unhappily we have deplorably little experimental evidence to lead us to any conclusion as to their functional significance in the cell economy. Our curiosity has been so insistent that we have resorted to less reliable sources of information, one of which is the argument from analogy. It is said that since the mitochondria are found in almost all active cells, their function must be a generalized one which they all possess in common. The validity of this line of reasoning rests entirely upon the resemblance of mitochondria in these different cells. Mitochondria, however, differ slightly in their solubility in acetic acid and in other respects, and in direct proportion to the extent of variation the above argument loses force.

[1] This work was also carried on at the Marine Biological Laboratory, Woods Hole, Mass., where the Director, Dr. Lillie, very kindly placed a research room at my disposal. It was aided by the Carnegie Institution of Washington.

A very important issue at stake is the relationship of plant and animal mitochondria. Are the researches of the botanist of interest to the anatomist and vice versa? Should we attempt to coördinate and correlate the two, and, if so, how far can we go? Though these questions have never been directly attacked, considerable difference of opinion is apparent in the literature. Suffice it to say that Pensa ('14, p. 22) states that the mitochondria in plant and animal cells are not identical; Guilliermond ('13*a*, p. 481) and Smirnow ('06, p. 153) think that they are; Duesberg and Hoven ('10, p. 99) believe them to be homologous; while Sapĕhin ('15, p. 320) holds an intermediate view. He thinks that in the higher plants mitochondria-like forms may be divided into two groups: "plastids" and "chondriosomes" which, however, are indistinguishable in the early meristem.

I have devoted my whole attention to this single problem of the relationship of plant and animal mitochondria with the hope of obtaining results which are clear-cut, concise and definite, bringing to bear upon it old, as well as entirely new, methods of technique in the form of supravital dyes of the janus green series which have never before been applied to plant cells.

Observations.

The statements concerning the similarity or the dissimilarity of plant and animal mitochondria have been made, with but few notable exceptions, by investigators having personal experi-

Figs. 1 and 2. Cells from the pea and the pancreas fixed in formalin and bichromate, mordanted in bichromate (Regaud IV. *B*) and stained with iron hematoxylin. (1,500 diameters.)

ence with one or the other, seldom with both. It is essential that the two should be compared side by side.

I have chosen for my experiments the cells of the radicle of the pea and the acinus cells of the pancreas of the mouse on account of the close resemblance of their mitochondria. The general appearance may be seen by reference to Figs. 1 and 2. Morphologically the mitochondria would seem to be identical in the two; but in reality this is not the case, for I have examined them very carefully with a high magnification (3,500 diameters) and I find that they are, on the whole, slightly longer and thicker

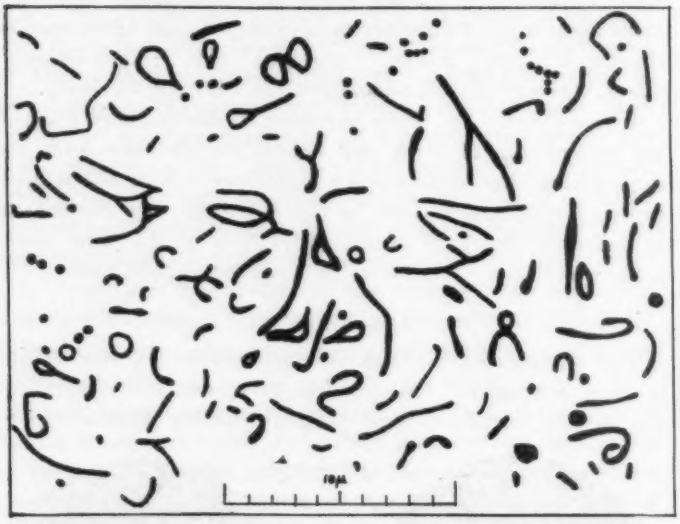


FIG. 3 Selected mitochondria from cells of pea radicle prepared by Regaud IVB to show the extent and limits of the polymorphism. (3,500 diameters.)

in the pancreas. I have made detailed drawings to scale of selected mitochondria from both. This can be done with considerable accuracy by making use of a good camera lucida, with appropriate illumination, and a sharply pointed pencil. The results are illustrated in Figs. 3 and 4. Filaments, rods and granules predominate. Branching filaments, networks, spherules and so on are rarer, but occur in both. In fact no form, however bizarre, is to be found in the one for which a counterpart cannot

be discovered in the other. The absence of very minute mitochondria, merging into the invisible, is of interest from the point of view of an origin *de novo* through condensation; and the polymorphism, in both the pea and the pancreas, makes plain the hopelessness of any attempt to devise a system of individual nomenclature, based upon morphology, to embrace all the forms. Obviously the material selected permits of very close comparison.

I have taken precautions in the following experiments to eliminate, as far as possible, chance variations due to incurrent

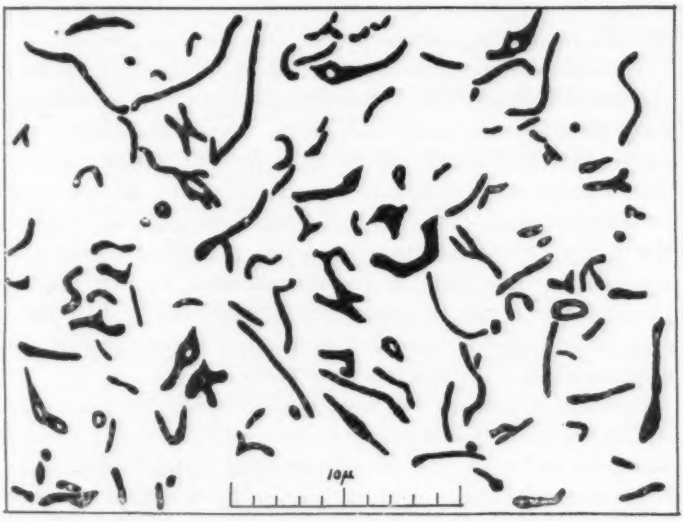


FIG. 4. Selected mitochondria from acinus cells of the mouse, treated in exactly the same way, showing similar polymorphism. (3,500 diameters.)

experimental errors which would, however, in any event, be likely to effect both tissues similarly (see p. 207). They were fixed together in the same bottle, dehydrated together, cleared together and embedded in the same block of paraffin together. They were cut with the same sweep of the knife, mounted on the same slide and stained at the same time. Many preparations have been made and in each of them the dehydration, clearing and embedding were carried on in the same way, so that the results are comparable. The sections were stained with iron

hematoxylin, with fuchsin methyl green, with Altmann's fuchsin picric acid and sometimes by the Benda method. The chief results are briefly set forth in the following table, but a large number of additional controls were made.

In a general way, running our eyes down the table, we see that the response of mitochondria to fixatives is the same in plants as in animals. Similar fixations preserve them, modify them and destroy them in much the same way in both. It also shows that the technique is easy, not difficult, and that a number of chemicals, which are generally thought to be destructive, will fix them more or less satisfactorily.

The standard mitochondrial fixatives, like Regaud ('10, p. 296) II., III., IV. *A* and IV. *B*, Benda's modification of Flemming's fluid, the acetic acid osmic bichromate mixture of Bensley, and Zenker, without acetic, will fix them in both tissues. I have found, however, that Regaud's mixtures are by far the most satisfactory because they do not produce the artificial coagulation of the ground substance caused by the others. They also preserve the true form of the mitochondria more faithfully; though I have reason to suspect that in some cases, in plant cells, they cause fragmentation and shrinkage. I have studied the behavior of the ingredients of these fluids.

Formalin is perhaps the most important since it penetrates rapidly, retains the homogeneous appearance of the ground substance and preserves the mitochondria satisfactorily. Formalin alone (Sapĕhin, '15, p. 321) serves as an excellent fixative for mitochondria. My best results were obtained with a solution of formalin of from 5 to 10 per cent. made up from ordinary commercial formalin neutralized with magnesium carbonate. More dilute solutions tend to cause a swelling of the mitochondria and more concentrated ones a shrinkage; but different tissues require different concentrations. Regaud advises a subsequent mordanting with bichromate, but I find that this is quite unnecessary. Used in combination with 3 per cent. potassium bichromate, according to the directions of Regaud, formalin gives the best of fixations with both the pea and the pancreas, though in my experience the results may be even improved by diluting the mixture with an equal volume of water as advised

TABLE I.[1]

No.	Fixative.	Radicle of Pea.	Pancreatic Acini.
1615B..	5% formalin 41 days	+normal	+normal
1574A..	5% " 4 "	+normal	+normal
1575A..	10% " 4 "	+normal	+normal
1616A..	40% " 4 "	+irregular, shrunken	+nodular, shrunken
1580....	2.5% " 4 " mordanted in 3% bichromate for 21 days	+but slightly swollen	+swollen and fragmented
1574....	5% "	+normal	+normal
1575....	10% "	+fragmented	+few
1576....	20% "	+normal	+few
1616B..	40% "	+normal	+normal
1585....	formalin 10 c.c., saturated aq. picric acid 40 c.c. (Regaud II) 1 day	+fragmented	+normal, indistinct.
1585B..	same, mordanted in 3% bichromate 20 days (Regaud III)	+normal, unusually filamentous	+normal, indistinct
1577A..	5% formalin, 10 c.c., sat. aq. picric acid 40 c.c., 1 day	+fragmented, distorted	+fragmented
1577....	same, mordanted in 3% bichromate for 16 days	+fragmented, distorted	−
1578....	10% formalin, 15 c.c., 1% chromic, 85 c.c.; mordanted in pyroligneous 1 day and 2 % bichromate 1 day	+fragmented	+few and fragmented
1566....	formalin 20 c.c., 3% bichromate, 80 c.c.; mordanted in 3% bichromate 15 days. (Regaud IV*B*)	+normal	+normal
1628 ...	Formalin 10 c.c., sat. aq. corrosive sublimate 40 c.c. 1 day	−	−
1629....	Formalin 10 c.c., sat. aq. corrosive sublimate 20 c.c., sat. aq. picric acid 20 c.c. 1 day	−	−
1582....	Boiling water 5 min.	−	−
1583....	95% alcohol	−	−
1447....	Gradual increase in concentration of alcohol, 2.5, 5, 7.5, 10, 15, 20, 30, 40, 50, 70, 80, 95% and absolute; chloroform	−	−
1584....	95% alcohol 10 c.c., 3% bichromate 10 c.c. 4 days	−	−
1618....	2% osmic 2 c.c. 2.5% bichromate 8 c.c., acetic 1 drop	+indistinct	+normal
1609....	sat. alcohol sol. corrosive sublimate	?	−

" + " signifies present, " − " absent and " ? " doubtful.

TABLE I—*Continued*.

No.	Fixative.	Radicle of Pea.	Pancreatic Acini.
1610....	sat. aq. corrosive sublimate	+distinct, shrunken	+few, indistinct
1611A..	sat. aq. picric acid	+nearly normal	+few, fragmented
1612....	2% chromic acid	+somewhat distorted	+few, fragmented
1613....	3% bichromate	+normal	+nodular rods, rings, granules
1614....	.5% osmic acid	+unsatisfactory	+normal

by Sapĕhin ('15, p. 321). Formalin in combination with picric acid is a somewhat erratic fixative, very excellent in some instances and bad in others. In good preparations the mitochondria appear very large and not so shrunken as when acted on by other fixatives. Formalin and chromic acid constitute a poor fixative which fragments the mitochondria and, in some cases, destroys them entirely.

Potassium bichromate used alone (Sapĕhin, '15, p. 321) is a good preservative for mitochondria but penetrates badly. Regaud recommends its use in mixtures and as a mordant in order to render the mitochondria insoluble in the alcohols during dehydration. The bichromate which remains in the tissue when it is sectioned, increases its affinity for fuchsin when the fuchsin methyl green stain is applied.

Ethyl alcohol in 95 per cent. solution destroys the mitochondria in both the pea radicle and the pancreas. When alcohol is employed in gradually increasing concentration the same result is obtained. When it is combined with potassium bichromate the mitochondria are also destroyed. In fact our conclusion can be none other than that alcohol, in whatever combination, is a very poor fixative for mitochondria.

Boiling water preserves the general form relations but does not bring to light any mitochondria.

Picric acid is a fairly good fixative for mitochondria in the cells of the pea and pancreas, when used in a saturated aqueous solution. It has the great advantage that it penetrates well.

Chromic acid alone causes considerable distortion of the mitochondria and somewhat irregular coagulation of the ground substance but in combination it is apparently very efficacious as shown by its inclusion in Benda's modification of Flemming's fluid and in other mixtures.

Osmic acid is the best preservative of mitochondria and the worst penetrator and is therefore useless in the cells of the pea radicle on account of their impermeable cellulose walls; yet it is this quality of being an excellent preservative which makes it so valuable in association with other chemicals. It does not cause any artificial coagulation of the ground substance.

Corrosive sublimate in saturated aqueous solution also penetrates the pea radicle very poorly; the pancrease not so badly. However it preserves the mitochondria in the pea far better than in the pancreas in spite of the fact that they are, in both cases, somewhat shrunken. In alcoholic solution it is destructive, but it is difficult to tell whether this is due to the alcohol or to the increased amount of sublimate. In Zenker's fluid the injurious action of the sublimate is counter-balanced by the presence of potassium bichromate.

The action of acetic acid is so important that I have made a detailed study of it as set forth in Table II.

In the first place I have varied the concentration of acetic acid in Regaud's mixture IV. *B* from 0 per cent. to 10 per cent. With no acetic acid the mitochondria are normal; while with a concentration of 2.5 per cent., or more, they are destroyed in both tissues, showing that their degree of solubility is the same.

I have also experimented with Zenker's fluid and I find that, with it, the mitochondria are on the whole much more resistant to acetic acid. This may be due to the fact that the Zenker's fluid was only applied for one day, instead of for five days like the Regaud's fluid; or the presence of sublimate in the Zenker's fluid may have tended to counteract the action of the acetic acid. The mitochondria are fairly well preserved when no acetic acid has been added to the Zenker's fluid. With increase in acetic acid, up to 10 per cent., they become destroyed in the deeper parts of the tissue, especially in the pea; while they are fairly well preserved in the most superficial cells in both. In the pea, also, the mitochondria are far more prone to become fragmented. This difference between surface and interior seems to be a question of penetration rather than of a difference in the mitochondria themselves. There is a simultaneous action of all the ingredients of the fixative on the cells at the surface and here the

TABLE II.

No.	Fixative.	Radicle of Pea.	Pancreatic Acini.
1570....	Zenker 0% acetic 1 day	+better preserved in older cells, apt to be vesicular, fragmented	+normal
1571....	" 2.5% "	+better preserved in older cells, apt to be vesicular, fragmented	+normal in peripheral cells, absent in center of tissue
1572....	" 5% "	+almost all destroyed in younger cells, still fairly numerous in older ones	+fewer, only in peripheral cells slightly fragmented
1573....	" 10% "	+more fragmented and vesicular	+same
1617....	" 20% "	+fairly well shown in all cells	+slightly fragmented, present in all cells
1627....	" 33% "	+but very rare	+but very rare
1626....	" 50% "	−	−
1566....	Regaud IV*B*, 0% acetic 5 days, mordanted in 3% bichromate 8 days	+normal	+normal
1567....	Regaud 2.5% acetic 5 day	−	−
1568....	" 5% "	−	−
1569....	" 10% "	−	−
1625....	Equal parts acetic and water	−	−
1624....	Pure acetic	−	−
1565....	Benda no acetic 8 days	+rather vesicular	+normal
1579....	Benda, 0.8% acetic, mordanted in formalin 20 c.c.; 3% bichromate 80 c.c. 6 days	+normal few	+normal
1579B..	Same but mordanted in pyroligneous 1 day and 2% bichromate 2 days	+normal few	+normal

bichromate, and possibly also the sublimate, modify the action of the acetic acid. In the deeper layers of the tissue, on the other hand, we have to do with a successive action of the said ingredients, determined by their relative rates of penetration. The acetic acid penetrates first and acts upon the mitochondria before the bichromate and the sublimate have come upon the scene.

Mitochondria are surprisingly resistant to higher concentrations. They are well preserved by a mixture of Zenker's fluid and 20 per cent. acetic acid and their relations are shown in Figs. 5 and 6. With 33 per cent. acetic acid only a few scattered mitochondria remain, while they are completely destroyed by a mixture containing 50 per cent. of acetic acid.

Pure acetic acid in half concentration and in full concentration destroys the mitochondria in both tissues. But it must not be thought that acetic acid in small concentrations is to be avoided because I have found that Benda's fluid without acetic acid does not seem to act so well as with acetic acid, in the amount prescribed.

In other words acetic acid exercises a solvent action on mitochondria which is the same in plants as in animals and varies with the distance of the cells from the surface and with the

FIGS. 5 and 6. Cells from the pea and the pancreas fixed in Zenker's fluid containing 20 per cent. of acetic acid and stained with fuchsin and methyl green. (1,500 diameters.)

character of the other components of the fixative. There is reason to believe, further, that the resistance of mitochondria in certain young plant cells is less than in old ones, calling to mind the condition which obtains in the spermatogenesis of many animals where the mitochondria in the young cells are more susceptible to acetic acid than in the mature spermatozoa (Regaud, '08, p. 661); which comparison makes the resemblance of plant and animal mitochondria, in this respect, still closer.

I have attempted to ascertain the experimental error in the study of mitochondria in fixed tissues in plants and animals and to see whether it is the same in both.

The conditions of the experiment are not so important as the nature of the fixative but they must nevertheless be taken into consideration. Variations in the temperature (under say 46° C.) and the illumination of the tissue prior to fixation do not seem to influence the mitochondria for I have grown peas at room

temperature and in the refrigerator (8 to 12° C.); in bright light and in darkness without bringing about any noticeable change in their mitochondria. Some fixatives are rather unstable and cannot be kept, even for a few hours, in bright sunlight or in a warm place. Under these conditions Regaud's formalin and bichromate mixture, for example, undergoes a rapid change characterized by a darkening in color, but its action does not seem to be impaired, though, to be on the safe side, one should avoid it. On the other hand, mechanical manipulation of the tissue before fixation often causes very confusing alterations in the mitochondria especially in the softer animal tissues.

The distribution of mitochondria within the cell is not altered appreciably in either plants or animals by the technique used, except in instances where mitochondria are present with different solubility, some being preserved and others being destroyed. This is of common occurrence in the pea radicle where with some fixatives, filaments only appear; the granules being obliterated so that the apparent distribution is altered.

The technique makes a very great difference in the number of mitochondria in the preparation. Some ingredients of the fixative are particularly likely to destroy them, like the acetic acid already mentioned; so that in the study of unfamiliar tissues we must assure ourselves that the technique is adapted to show all the mitochondria present.

The shape of mitochondria is so easily modified by the fixative that we must be on our guard here also, especially in plant cells. That is to say, we frequently meet with a fragmentation of mitochondrial filaments, the filaments of the living cell appearing in the form of rows of granules in the fixed preparation. The reverse change never takes place, for filaments are never formed through a coalescence of granules, under the influence of the fixative. Poor penetration of the fixative in the deeper layers of the tissue often causes the mitochondria to lose their characteristic form and to swell up into large spherules or vesicles. Similarly if a film on the surface of the tissue is allowed to dry, before fixation, the mitochondria in it will be profoundly modified.

The size of mitochondria is also subject to some modification, as shown by the comparison of mitochondria in living cells with

those in fixed tissues. The general tendency of fixatives is to shrink them and to make them uniformly smaller than they really are in the living condition. This tendency is very slight, almost negligible, in the case of the best fixatives. On the other hand, acetic acid, and possibly also formalin in very low concentrations, have a marked swelling effect.

It must be admitted that variations sometimes do occur in the mitochondria in our preparations, particularly in the pea, for which no reasonable explanation can be advanced on the basis of the technique. They may result from rhythmical variations in the activity of the cells which we have reason to believe occur, just like periodicity in cell division, many cells dividing almost synchronously (Kellicott, '04, p. 531) but they cannot be wholly explained in this way. They may result also from the conditions under which the radicle is growing, upon whether it is submerged or exposed to the air; but I find that radicles grown under exactly the same conditions occasionally show mitochondrial variations which are very perplexing.

Evidently the experimental error is something which we must have considerable respect for, but which can be controlled if the proper precautions are taken. Its identity in plant and animal tissues is another indication of the similarity of the mitochondria in the two.

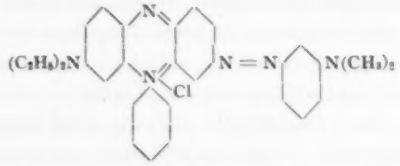
There is another factor, often very confusing, which we have to bear in mind, namely that of the production of coagula in the homogeneous ground substance. They are formed in the protoplasm of the pea radicle by exposure for about 30 minutes to a temperature of 48° C. or higher and also by fixatives containing acetic acid, chromic acid, corrosive sublimate or alcohol. Iron hematoxylin, when imperfectly differentiated with iron alum solution, stains these coagula in the same manner as the mitochondria and in many cases no clearly marked distinction can be made between them, since they both vary very much in their resistance to differentiation. The formation of these coagula is generally accompanied by distortion, fragmentation or destruction of the mitochondria. In the cells of the pancreas these fixatives bring about a distinctly fibrous appearance in the normally homogeneous basophilic substance which is often as re-

sistant to differentiation as are the mitochondria, but the destructive effect is not so apparent as in the cells of the pea radicle.

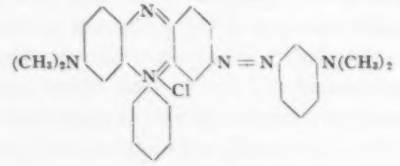
The question of the reactions of plant and animal mitochondria after fixation, to stains may be dismissed with very few words. When they have been fixed by any method, it is usually possible to color them with any stain, by the Benda method, with fuchsin-methyl-green, with fuchsin-picric acid or with iron hematoxylin, but it is sometimes advisable to select some mordant in order to induce the stain to take. The Benda method is the most difficult and it is sometimes refractory, for no apparent reason, in tissues where the others give excellent results. In other words there is nothing whatsoever specific about the staining reaction.

A number of supravital stains have been discovered for mitochondria in animal cells, of varying degrees of specificity, and it is interesting to note that none of them have heretofore been applied to plant mitochondria. The most delicate is the janus green reaction, which we owe primarily to Michaelis ('99, p. 565). The delicacy of this reaction is shown by E. V. Cowdry's ('16, p. 429) observation that janus green B will stain mitochondria specifically in human lymphocytes in a dilution of half a million (*i. e.*, one part of stain to 500,000 parts of salt solution), and that janus green G will not stain them even in a relatively strong solution.

Janus green B is diethylsafraninazodimethylanilin:



Janus green G is dimethylsafraninazodimethylanilin:



Janus green G differs only in the substitution of a *dimethyl* group in the place of the *diethyl* group in the safranin molecule. Janus blue and janus black I were first introduced as vital stains for mitochondria by E. V. Cowdry ('16, p. 431). Janus blue is the trade name for diethylsafranin-B-napthol and janus black I is a mixture of diethylsafraninazodimethylanilin and some other dye of unknown constitution. Diethylsafranin may easily be made from janus green by splitting off the azodimethylanilin group (Cowdry, '14, p. 269). I have applied these dyes to the mitochondria in the cells of the pea radicle and the acinus cells of the pancreas with the following results.

TABLE III.

Dye.	Cells of Pea Radicle.	Acinus Cells of Pancreas.
Janus green B	+ Intense	+ Intense
Janus green G	−	−
Janus blue	+	+
Janus black I	+	+
Diethylsafranin	?	? + faint
Nilblue B, extra	+ faint	+ faint

It will be seen that the mitochondria in plant cells react to vital stains in precisely the same way as in animal cells, even to this extraordinarily delicate janus green test. It is very much more difficult, however, to get a good stain in plant cells by reason of their tough, cellulose walls offering an almost insurmountable barrier against the penetration of the dye; and janus green is at best but a poor penetrator compared with neutral red, methylene blue and others. For this reason the staining of mitochondria is much slower than in animal cells. Great difficulty was also experienced in finding a suitable medium for the examination of the two tissues. At first they were examined, side by side, in weak aqueous solutions of the dyes to which sodium chloride had been added in varying amounts. This proved entirely satisfactory for the pancreas, but failed with the pea, for which a sugar solution was used. Another trouble with the pea, not encountered with the pancreas, is that the cells are cemented closely together and are therefore so very difficult to separate by teasing, that thin sections of the living pea radicle had to be cut with a razor blade. This method also is objection-

able because a comparatively large amount of the tissue juice is liberated which brings about a troublesome precipitate of the dye and makes it necessary to renew it frequently. In short, plant cells are very hard to handle but the stain is none the less specific. The petals of some flowers (narcissus, sweet pea, freesia, etc.) give very much more satisfactory results than the pea since with them the dyes penetrate more easily.

I have also tried to stain the living plants *in toto* by growing them in sand moistened with a strong solution of janus green B. I discovered at once that they react differently to this treatment. Peas were stunted, but grew and flowered without any coloration with the exception of the root hairs and small portions of the epidermis which seemed to be dead. On the other hand, gourds grew vigorously for a time and became intensely stained while growing. They presented a very beautiful appearance because in some of the tissues the dye is reduced to its color base, diethylsafranin, so that the plant is marked off into red and bluish green areas following the distribution of processes of reduction and of oxidation. Cells from the different regions were examined and it was found that the whole protoplasm was stained more or less uniformly and that the mitochondria were not specifically colored. These experiments would seem to indicate that the vital staining of plants with the less toxic azo dyes is full of promise from the point of view of plant physiology.

This specific comparison of the mitochondria in the pea and in the pancreas has shown that morphologically they are almost identical, save for a slight difference in diameter. No forms are present in the one for which counterparts cannot be discovered in the other. If they could be viewed dissociated from their environment it would be next to impossible to tell which belonged to the pea and which to the pancreas. Microchemically they are identical so far as our imperfect methods go. Similar fixatives preserve them, modify them and destroy them in like fashion in both. Even the experimental error is the same. Finally, and most important of all, they react the same way to the janus green test and to other supravital dyes. Accordingly our provisional conclusion can be none other than that the mitochondria in the pea and in the pancreas are composed of precisely the same material.

But this is only a single isolated instance and many facts of interest can be brought to light by a broad general discussion of mitochondria in many forms which I shall now venture to make, based in part upon the literature.

Discussion.

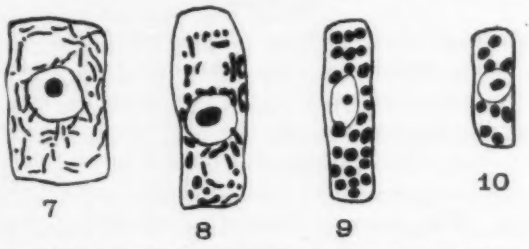
Distribution.

In plants mitochondria have been recorded from the *Angiosperms* to the *Fungi;* but it is difficult or even impossible to demonstrate their existence in the very lowest forms of plant life, like the *Myxomycetes*, the *Schizomycetes* and some of the *Algæ;* though in these groups structures of questionable nature have been discovered which may ultimately prove to be mitochondrial. This absence of typical mitochondria in the lowest plants may be contrasted with their almost universal occurrence in the *Protozoa*. In all forms of animals, from amœba to man, which have been investigated with adequate methods of technique, they occur without exception.

With regard to the different types of cells. In plants, they occur from the tip of the root to the end of the stem, wherever the protoplasm is active, with but few exceptions. The same is true in the various categories of animal cells. They are met with in gland cells, nerve cells and muscle cells; in connective tissue cells, germ cells and almost all others, except in the terminal stages of cytomorphosis. And this is one of the greatest points of similarity between these granulations in the plant and animal kingdoms, that it to say their progressive diminution and final absence in the later stages in the life of the cell.

I refer, for instance, to the decrease in number of mitochondria in plant cells, which runs parallel to the formation of chloroplasts, for it is said (Guilliermond, '12, plates 17–18) that when the plasts are fully mature few if any mitochondria remain (which reminds one of the single large chloroplast and the absence of mitochondria in some algæ); and these are mature and highly differentiated cells. In animals there is a similar disappearance of mitochondria in the life cycle of red blood cells. In the young, nucleated forms they are very abundant, they become less and less so as the cell differentiates; a few persist after the nucleus is

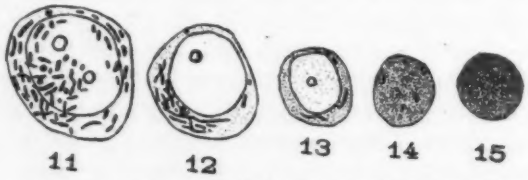
lost; but in the fully mature forms, as they occur in the circulation in man, mitochondria are entirely absent. In plants this disappearance is associated with the production of chlorophyll; in animals, with the formation of hemoglobin, two substances with strikingly similar chemical constitution; in both it is progressive and runs parallel with an increase in the degree of



FIGS. 7, 8, 9 and 10. Meristem and parenchyma cells of the bean (after Guilliermond, '12, plate 18, modified) showing the progressive disappearance of mitochondria with the formation of plastids containing chlorophyll.

differentiation and with the age of the cell, general metabolism being diminished and special functions being accentuated (Figs. 7–15).

This diminution of mitochondria in cytomorphosis is really of very common occurrence but it attracts attention only in those



FIGS. 11, 12, 13, 14 and 15. Erythroblast, megaloblast, normoblast and erythrocytes from bone marrow of a rabbit stained vitally with janus green showing the parallelism between the disappearance of mitochondria and the appearance of hemoglobin in the form of a diffuse deposit. (1,500 diameters.)

cells which normally die and are replaced in large numbers, collectively, in the life of the organism.

In the separate regions of the cell, also, there is a general similarity in the distribution of mitochondria in plants and animals. Where the cells are elongated, as in the plerome,

filamentous mitochondria are usually distributed parallel to the long axis; which calls to mind the arrangement of mitochondria in gland cells, nerve cells, muscle cells and others. In the serous cells of the parotid they are heaped up in the proximal region next the basement membrane and remote from the lumen (Fig. 16). Where the polarity is reversed as in the thyroid (Fig. 17), the mitochondria are also reversed; while in the epithelial cells of the intestine, they are condensed at both poles (Fig. 18). This, Champy ('12, p. 109) thinks, is indicative of a double polarization for adsorption and for secretion. Corresponding condensations have not been recorded in plant cells.

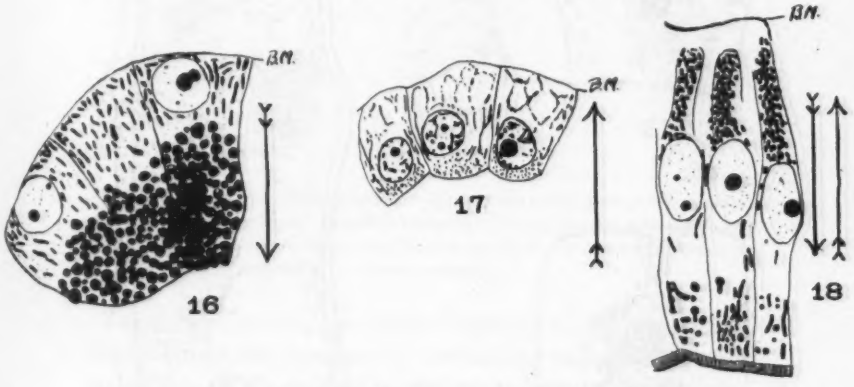


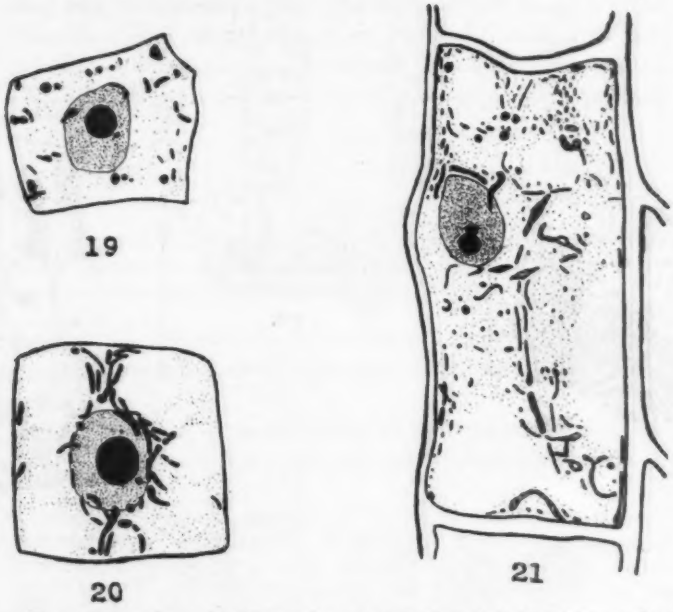
FIG. 16. Serous cells of parotid of mouse with proximo-distal polarity, as represented by the arrow, B. M. being the basement membrane. (1,500 diameters.)

FIG. 17. Thyroid cells (after Bensley, '16, p. 55) with reversed polarity, disto-proximal, in the direction of the basement membrane.

FIG. 18. Intestinal epithelial cells with double polarity, mitochondria being accumulated at both poles. (1,500 diameters.)

Mitochondria, however, group themselves about the nucleus in both plants and animals. In the early meristem of plants, generally, mitochondria are found indifferently distributed in the protoplasm (Fig. 19). They soon approach and appear to come into actual contact with the nucleus (Fig. 20) in which position they enlarge and form plasts which migrate away from the nucleus and become distributed more or less evenly in the surrounding protoplasm (Fig. 21). Guilliermond has repeatedly described this migration. I find that the mitochondria become

progressively more resistant to acetic acid in this process of plast formation. Similarly in the spermatogonia of certain animals the mitochondria are diffusely arranged (Fig. 22); in the spermatocytes they approach the nucleus (Fig. 23) and become so closely applied to it that investigators have been deluded into thinking that they actually originate from it. In the later stages of spermatogenesis they leave the nucleus (Fig. 24)



FIGS. 19, 20 and 21. Meristem and young and old cortical cells of the pea fixed in formalin and picric acid (Regaud III) and stained with iron hematoxylin showing primary diffuse arrangement of mitochondria, secondary condensation about the nucleus and final even distribution throughout the whole cytoplasm. (1,500 diameters.)

becoming more resistant to acetic acid as Regaud ('08, p. 662) has shown. Indeed the parallelism is very close. Other instances might be cited.

In plant cells one rarely finds a condensation of mitochondria in the peripheral cytoplasm though it is frequently met with in animal cells. It is particularly manifest in certain stages in the

growth and maturation of the egg as Van der Stricht ('09, plate 1) and his pupils, among others, have abundantly shown. The causes underlying these strange movements of mitochondria are obscure.

Furthermore, during cell division the mitochondria are distributed in much the same way in plants as in animals. They persist during the whole process; they are absent in the spindle area, whether a definite spindle be formed or not; and they are divided in approximately equal amounts between the two daughter cells.

FIGS. 22, 23 and 24. Spermatogonium, spermatocyte and spermatid from mouse testis fixed in formalin and bichromate (Regaud IV*B*) and stained with iron hematoxylin. Note diffuse arrangement of mitochondria, circumnuclear condensation and final diffuse arrangement. (1,500 diameters.)

In animal cells they are almost invariably disposed in a radial fashion about the centrosome: contrasting strongly with their appearance in *Pustularia* during spore formation when they are entirely absent from the region near the centrosome and are found in a clump in the portion of the cells farthest away from it (Guilliermond, '13*b*, p. 649). No examples of a radial arrangement have to the best of my knowledge been described in the higher plants but this may be due to the well-known absence of a typical centrosome in the *Angiosperms*.

It will be noticed that in animal cells rather more variations obtain in the arrangement of mitochondria than in plant cells, but this seems to be correlated in some way with the fact that animal cells are more generally polarized. I mean polarized for irritability, conduction, secretion, contraction and so forth, properties which do not play so great a rôle in the life of plants, where separate regions of the cell are not so distinctly marked off. Accordingly if one were searching for variations in the distribu-

tion of mitochondria in different parts of plant cells one would be inclined to examine cells polarized with reference to light, secretion and so on, where cytoplasmic division of labor may be expected.

Morphology.

As we pass down the plant scale we find no noteworthy differences in the morphology of mitochondria, even in those *Algæ* which possess them they are alike. Similarly, few animals have mitochondria of distinctive morphology, though in certain species of both plants and animals, either filamentous or granular forms may predominate.

In the different tissues of plants there is some variation in the size and shape of mitochondria. In some cells thick filaments are most abundant, while in other kinds of cells, granular forms of variable size may be found. Networks are rarely met with. The question is complicated by the fact that in certain tissues all morphological transitions between typical mitochondria and true plastids are to be seen; swellings develop in the region of the mitochondrial filaments, which apparently grow larger and larger and ultimately form mature plastids.

In the different tissues of animals there is rather more variation in the morphology of mitochondria. They are usually filamentous in gland cells, rod-like in muscle cells and they are often granular in egg cells. Networks occasionally occur in the pancreas and in spermatogonia as well as in other locations. Even within gland cells of the zymogenic type there is considerable variation. The mitochondria in the acinus cells of the pancreas are uniformly longer and thicker than those in the chief cells of the fundus of the stomach and I find that they are two or three times as long as those in the serous cells of the parotid of the mouse (compare Figs. 2 and 16). The mitochondria in zymogenic cells often possess little enlargements which are supposed to be the precursors of granules of zymogen, and which call to mind the swellings on the mitochondria in plant cells during plastid formation. The similarity of the process may be seen by taking two specific instances; the production of metachromatic corpuscles in plants as figured by

Guilliermond ('13*c*, p. 438) and the formation of fat as described by Dubreuil ('13, p. 104*B*). Compare Figs. 25 and 26.

Striking differences also obtain in the morphology of mitochondria in the different categories of nerve cells. Nicholson ('16, p. 347) has found that they are usually filamentous, especially in the anterior horn cells and in the cells of the reticular formation; they are rod-like and granular in the large and small cells of the Gasserian ganglion, and they occur in the form of large irregular blocks in the cells of the trapezoid nucleus.

25 26

FIG. 25. Metachromatic corpuscles developing in mitochondrial filaments (after Guilliermond, '13*c*, p. 438).

FIG. 26. Droplets of fat forming in mitochondrial filaments (after Dubreuil, 13, p. 104).

The greater polymorphism of animal cells, with regard to their mitochondrial content, probably results from the fact that their functions are more diversified, for they have a greater variety of duties to perform under different conditions. Apparently their distribution within the cell is more varied in animals than in plants for the same reason (p. 212). The form of mitochondria is apparently independent of the degree of mobility of the surrounding protoplasm.

It is worthy of note that in developing tissues of both plants and animals similar and parallel changes take place in the morphology of mitochondria, at least in certain instances. In the spores of plants and the egg cells of animals, they are usually granular and sometimes filamentous; in the developing tissues of the embryo they are usually filamentous and rarely if ever granular. With the assumption of special functions on the part of the cells in the different organs they change their form. Some remain filamentous and acquire blebs (secreting cells), others become more rod-like (muscle), still others granular, and so on.

A single cell may contain all the types of mitochondria which have been enumerated. This is true for animals as well as for

plants. Generally, however, filamentous, rod-like, dumb-bell and granular forms are met with. Filaments, when present, have an astonishingly uniform diameter in the same cell, though they may possess the swellings already mentioned. Segmentation of filaments is of common occurrence in both and it is possible that a coalescence of granules may take place. In some tissues there is considerable variation from cell to cell, while in others the morphology of the mitochondria is quite uniform throughout. Thus the "anthocyane" producing mitochondria, figured by Guilliermond ('13*a*, p. 479), are uniform; those in the cortical cells of the pea radicle, variable in length. In neighboring acinus cells of the pancreas of the mouse they are uniform; in contiguous spinal ganglion cells, variable.

A fundamental distinction may be made between variations in length and variations in breadth, in the case of both plant and animal mitochondria, to which I have seen no reference in the literature. While the mitochondria in the cells of the pea radicle vary greatly in length, filamentous and rod-like forms predominating, their girth is remarkably uniform, throughout the whole tissue, in individual cells and in different parts of the same filament (Fig. 1). The same holds for the pancreas (Fig. 2). The uniformity in girth extends right to the end of the filaments which always end abruptly without tapering. The truth of this statement is made still more apparent by examining any illustration of mitochondria, because artists are wont, quite rightly, to draw each filament with a single sweep of the same pen or brush. The one great exception to this rule is plastid formation in plants and the production of bleb-like swellings in association with secretion in animal cells. The diameter of the mitochondrial filament is fixed, but the length is not; one is stamped on the cell through its organization and is probably dependent on the water content of the surrounding cytoplasm, the other is most likely an expression of growth by accretion affecting the length but not the diameter. We have here two attributes, independently variable, which may perhaps be influenced in different ways, common to both plant and animal mitochondria, which speak more strongly for their identity than any other.

Composition.

We must bear in mind the unsatisfactory nature of the evidence upon which our views of the chemical constitution of mitochondria are based. It is impossible to make a direct chemical analysis of them. We are forced to use solvents, special stains and so forth. We know astonishingly little positively, but we infer a great deal, often on very insufficient grounds. Most of the work has been done with animal cells, and investigators have arrived at the general conclusion that here mitochondria are of the nature of phosphatids, in other words, that they contain fatty acid, phosphoric acid, glycerol and some nitrogen base. Lecithin is an example of a typical phosphatid and has been used to manufacture artificial mitochondria. Briefly the evidence is as follows:

1. In animal cells it has been found that mitochondria are almost completely soluble in alcohol, ether, chloroform and dilute acetic acid. They are rendered insoluble by chromization, and also, in my experience, by treatment with formalin, at least in some cases. They do not stain with Sudan III. or Scharlach R. They are only sometimes blackened with osmic acid.

In plant cells they are also soluble in alcohol, ether, chloroform and dilute acetic acid and they are here rendered insoluble by chromization in exactly the same way. Treatment with formalin also makes the mitochondria insoluble. The whole cell blackens with osmic acid so readily that it is impossible to ascertain just how strongly the mitochondria themselves react to it.

2. It is said, in the literature, that part of the mitochondrial substance, in animal cells, is not soluble in these fat solvents and it has been assumed that this portion is albumin. The results of applying Millon's reagent are uncertain and difficult to interpret.

In view of the statement of Forbes and Keith ('14, p. 73) that vegetable phosphatids differ from those in animal cells in that they nearly always contain sugar firmly bound to the rest of the molecule, we should bear in mind the possibility of sugar being a likely constituent of mitochondria in plants.

3. Artificial mitochondria have been made by Löwschin ('13, p. 203) of lecithin and albumin solutions which apparently

present the same form and solubilities as true mitochondria in both plant and animal cells. They form granules, rods and filaments which multiply by division. He embedded them in glycerin-gelatin, fixed them and found that they stained in the usual way by the various mitochondrial methods.

I have myself experimented with lecithin. I made a very fine emulsion, evaporated it to dryness on glass slides and stained with iron hematoxylin. The bodies which I observed resembled mitochondria in some respects but in others differed so very radically from them that I am unable to confirm Löwschin's observations.

4. The temperature solubility of mitochondria may be also significant because mitochondria, like phosphatids, are thought to have a low dissolving point. Policard ('12, p. 229) observed, in the case of some animal tissues, that the mitochondria are dissolved when subjected to a temperature of from 48° C. to 50° C. in a moist atmosphere from 10 to 30 minutes, while the other parts of the cells remain practically unaffected; and Koch and Voegtlin ('16, p. 59) remark that phosphatids are notoriously unstable to heat. With the hope that this temperature solubility would turn out to be a definite physical test for mitochondria, I experimented with both the pea and the pancreas, observing the precautions outlined on page 199. I found that in both the pea and the pancreas they dissolve at about the same temperature, that is to say, from 48° to 50° C., when treated for 30 minutes. The first change noted is a loss of their filamentous form (see also the Lewises, '15, p. 375); they became granular with indistinct outlines merging into the surrounding cytoplasm and they finally disappeared leaving no trace behind. In the case of the pea, the mitochondrial changes are difficult to make out on account of the production of a confusing coagulation of the ground substance.

5. The specific gravity of mitochondria is generally greater than that of the protoplasm in which they are embedded (Fauré-Fremiet, '13, p. 602). This is determined by the unsatisfactory centrifuge method. When they are thrown down they are said to be of high specific gravity. If the protoplasm is in the physical condition of a gel, rather than a sol, as in the nerve cell, the dis-

tribution of mitochondria is unaltered by centrifuging, as Key discovered. But this is no reason to believe that these mitochondria differ from others in their specific gravity. At any rate, where the method is applicable (*i. e.*, in egg cells) the mitochondria are heavier than protoplasm, in which respect they conform to what we know of phosphatids and differ sharply from oils and neutral fats which rise to the surface and float, instead of being thrown down.

6. In some animal cells mitochondria act as solutes for different substances. Pigments of various kinds are frequently found dissolved in their substance so that they assume the most brilliant hues. Asvadourova ('13, p. 263), following Prenant, calls them "chromochondria" on this account. The presence of droplets of neutral fat within the mitochondrial filaments has also been recorded.

Plant mitochondria behave in exactly the same way, anthocyanin, chlorophyll and other pigments, sugar, starch and even fat being heaped up within them (Guilliermond, '13*b*, p. 647).

7. There seems to be a certain correspondence between variations in the histological picture of mitochondria and variations in the phospholipin content of the same organ on chemical analysis. Thus Mayer, Rathery and Schaeffer ('14, p. 612) have been able to alter the mitochondria experimentally in liver cells. In stages with more mitochondrial substance, chemical analyses show an increase in phosphorized lipoid; in stages with less, diminution.

Unfortunately mitochondria in plant cells have not been investigated from this point of view.

8. And, finally, the very interesting observations of Russo and Rene Van der Stricht must be mentioned. Russo ('12, p. 215) claims to have been able to increase the number of mitochondria in the oöcytes of the fowl by the injection of lecithin and Rene Van der Stricht ('11, p. 435) obtained results which seemed to be confirmatory.

Since there have been no observations along this line in plant cells I have attacked the problem by growing peas in solutions of lecithin. The results were in no sense definite and concise, though the mitochondria did seem to be increased in diameter.

Physiology.

It is generally conceded that mitochondria in plant cells play an important part in the elaboration of chlorophyll and starch. They are thought to do this through the intermediary of the chloroplasts. According to Guilliermond ('12, p. 387) chlorophyll appears in typical mitochondria, increases in amount, other changes take place and the mature chloroplast results. It is not surprising, therefore, that mitochondria have attracted so much attention among European botanists, because the formation of these substances had been under discussion for nearly a century and a deadlock had been reached before these new mitochondrial methods were devised. Indeed the formation indirectly of starch from atmospheric carbon dioxide and water, through the action of sunlight on chlorophyll, is the most fundamental of all vital processes in plants. Mitochondria are concerned in the formation of chlorophyll and thus the very existence of the plant depends on them. Plants furnish the food of animals so that the importance of mitochondria with respect to the food supply is apparent.

Very obviously the mitochondria in typical animal cells can take part in no such process since there are no plastids, and starch is elaborated only in plants. But this is not, as it might seem to be, a fundamental distinction between the two, for many consider the animal mitochondria themselves to be plast-like and to act as such in the elaboration of secretions. The evidence for this, however, is not entirely conclusive and we must bear in mind, in all experimental work, the great importance of the homogeneous ground substance, or environment, in which the mitochondria are embedded. Apparent alterations may occur in the mitochondria which cannot be attributed to the mitochondria themselves, but only to changes in their environment. This is particularly true in the case of their refractive index. The mitochondria may stand out sharply in one cell and be quite invisible in another and yet be identical as far as their function is concerned in the two cells, for variations in the surrounding protoplasm may alone be responsible for the difference in appearance. There are thus two variables, the mitochondria and the protoplasm, either one of which may bring

about a visible difference in the living cell independently of the other, though in the majority of the cases they probably act together. So that it is very difficult to tell whether the mitochondria are active or passive agents.

The belief is gaining ground (Kingsbury, '12, p. 46; Mayer, Rathery and Schaeffer, '14, p. 619, and others) that mitochondria are concerned with respiration in animal cells; that is to say, with the taking up of oxygen. This conception is based upon the view that mitochondria chemically resemble phosphatids, it being thought by some that phosphatids outside the body are capable of auto-oxidation. It falls well in line with the very wide distribution of mitochondria as well as with the fundamental nature of the process of respiration. And of course, the same arguments apply to mitochondria in plant cells for plant cells also take up oxygen. It seems that, in this particular, there is no great difference between mitochondria in these two great groups of organisms.

Unfortunately it is not possible to transfer plant mitochondria to animal cells to see what they would do, or vice versa. We are working in the dark, but we have only ourselves to blame because we have not taken full advantage of certain lowly protozoans, like *Euglena viridis* and others, which have, confined in the scope of a single cell, all the properties which we are prone to consider distinctive of both plants and animals. They contain chlorophyll, produce paramylum, a carbohydrate resembling starch, and at the same time engulf, devour and digest certain still more minute organisms. The brief description of mitochondria-like material in *Euglena*, made by Ternetz ('12, p. 463), would serve as a point of departure. The planarian worm, *Convoluta roscoffensis*, which takes in algæ and lives in symbiotic relationship with them would repay further study. We have here an animal containing animal mitochondria with all their attributes which, after symbiotic relationship has been established, "ceases to take in solid food and depends entirely upon its vegetable partners" (Bayliss, '15, p. 295). Another opportunity is afforded by *Chlamydomyxa labyrinthuloides* which when at rest lives as a plant and when active like an animal (Parker and Haswell, '97, p. 49).

One point more. The close resemblance of mitochondria, amounting almost to identity in both plants and animals, may indicate a common ancestral form possessing mitochondria. It is a general assumption that plants antedate animals, and it seems significant that among the lower plants, and not in the lower animals, we find groups in which mitochondria have not been detected. I have particularly in mind the *Algæ*, for in this interesting group some forms contain mitochondria and others do not (M. and Mme. Moreau, '15, p. 730). The absence of mitochondria is interpreted in various ways. Some think that the large chloroplast takes over their function (Guilliermond, '13, p. 86) and it is possible that the mitochondrial substance may be present in an invisible diffuse condition. There is every reason to believe that the possession of formed mitochondria is an attribute even more primitive than the possession of a fully formed nucleus, which, unlike the mitochondria, has gradually assumed a more specialized character. Yeast plants in which the presence of a nucleus is debated (Macallum, '99, p. 67) unquestionably contain them (Janssens and Helsmortel, '13, p. 452), and there are indications that some bacteria also have mitochondrial material. This is beside the question, however, because the *Saccharomycetes* and *Schizomycetes* are probably degraded forms. We can look upon the ancient line of evolution as passing through forms closely resembling some of the *Algæ* of the present day which are devoid of mitochondria, into similar forms possessing mitochondria and which gradually acquired fully developed nuclei in addition. From these nucleated alga-like organisms containing mitochondria, the higher plants and the whole animal kingdom gradually evolved. They stood at the parting of the ways. The geological record, incomplete as it is, seems to indicate that the algæ are the oldest of plants; those of the present day being the lineal and comparatively unaltered descendants of the most ancient ones. It is surprising that mitochondria should persist without great modification through all the storm and stress of millions of years of evolution during which everything else changed except perhaps the primitive living homogeneous ground substance. As they were in the beginning so they are now, on the crest of both animal and plant

evolution, their form in *Vaucheria* (Rudolph, '12, Pl. 18, Fig. 9) being equally indistinguishable from that of the oak and of man. This paper shows how much alike they are everywhere. We are tempted to enquire whether the presence of mitochondria in conjunction with a nucleus made evolution possible. This may indeed be true if, as I have already mentioned (p. 223), they are concerned with protoplasmic respiration, which is perhaps the most fundamental of all vital manifestations. Certainly no consideration emphasizes their importance more.

Summary.

This comparison of mitochondria in plant and animal cells brings to light a truly remarkable degree of similarity.

Their *reactions to fixatives, stains and supravital dyes* are almost identical. Similar fixatives preserve them, modify them and destroy them, in like manner, in both. Even the result of experimental errors in the technique is the same. Plant mitochondria react to the janus green test and stain with supravital dyes in substantially the same way as animal mitochondria, though it is certainly more difficult to obtain a good coloration.

Their *distribution* is almost universal. They occur in all plants with the exception of certain *Algæ*, *Bacteria* and *Myxomycetes*, and no animal has yet been discovered which does not contain them. In addition to this, similar and parallel variations occur in their arrangement in the several tissues and even in the individual cells of plants and animals. They must, therefore, be regarded as an integral, perhaps essential, constituent of living matter.

Their *morphology* is identical in plants and in animals. They assume no forms in the one, which are not present in the other. They undergo similar variations in size and shape in different tissues and in different cells in both. If it were possible to view mitochondria dissociated from their environment, it would be impossible to decide whether they came from plant or animal tissues, provided that they did not contain starch, pigment or some other easily recognizable substance, to serve as a clue.

We have every reason to suppose that their chemical *composition* is much the same in both plants and animals, but here our

knowledge is for the most part supposition and inference, since direct chemical analyses are obviously out of the question. Their composition, as indicated by solubility with respect to acetic acid, heat and other agents, is certainly subject to similar variations in both.

Their *physiology* is obscure; but their wide occurrence in all protoplasm and their general similarity in all places must mean something. It may mean that, in addition to certain specific functions like the production of chlorophyll, they all have a common duty or part to play in some fundamental vital manifestation like protoplasmic respiration.

LITERATURE.

Asvadourova, Nina.

'13 Recherches sur la formation de quelques cellules pigmentaires et des pigments. Arch. d'Anat. Micr., T. 15, pp. 153–314.

Bayliss, W. M.

'15 Principles of General Physiology, London, Longmans, Green, and Co., 850 pp.

Bensley, R. R.

'16 The Normal Mode of Secretion in the Thyroid Gland. Amer. Jour. Anat., Vol. 19, pp. 37–54.

Champy, C.

'13 Recherches sur l'absorption intestinale et le rôle des mitochondries dans l'absorption et la sécrétion. Arch. d'Anat. Micr., T. 13, pp. 50–170.

Cowdry, E. V.

'14 The Vital Staining of Mitochondria with Janus Green and Diethylsafranin in Human Blood Cells. Intern. Monatsschr. f. Anat. u. Phys., Bd. 31, pp. 267–286.

'16 The General Functional Significance of Mitochondria. Amer. Jour. Anat. Vol. 19, pp. 423–446.

Dubreuil, G.

'13 Le chondriome et le dispositif de l'activité sécrétiore, etc. Arch. d'Anat. Micr., T. 15, pp. 53–151.

Duesberg, Jules and Henri Hoven.

'10 Observations sur la structure du protoplasme des cellules végétales. Anat. Anz., Bd. 36, pp. 96–100.

Fauré-Fremiet, E.

'13 Le cycle germinatif chez l'Ascaris megalocephala. Arch. d'Anat. Micr., T. 15, pp. 435–757.

Forbes, E. B. and Helen M. Keith.

'14 Phosphorus Compounds in Animal Metabolism. Ohio Agricultural Experiment Station, Wooster Technical Bulletin, No. 5, 748 pp.

Guilliermond, A.

'12 Recherches cytologiques sur le mode de formation de l'amidon et sur les plastes végétaux, etc. Arch. d'Anat. Micr., T. 14, pp. 309–428.

'13 Sur la signification du chromatophore des algues. C. Rend. Soc. Biol., T. 75, pp. 85–87.

'13a Quelques remarques nouvelles sur la formation des pigments anthocyaniques au sein des mitochondries. A propos d'une note récente de M. Pensa. C. Rend. Soc. Biol., Paris, T. 75, pp. 478–481.

'13b Nouvelles observations sur le chondriome de l'asque de Pustularia vesiculosa. C. Rend. Soc. Biol., Paris, T. 75, pp. 646–649.

'13c Nouvelles remarques sur la signification des plastes de W. Schimper par rapport aux mitochondries actuelles. C. Rend. Soc. Biol., Paris, T. 75, pp. 437–440.

Janssens, F. A. and J. Helsmortel.

'13 Le chondriome dans les Sacchyaromycètes. La Cellule, T. 28, pp. 451–452.

Kellicott, W. E.

'04 The Daily Periodicity of Cell Division and of Elongation in the Root of Allium. Bull. Torrey Bot. Club, Vol. 31, pp. 529–550.

Kingsbury, B. F.

'12 Cytoplasmic Fixation. Anat. Rec., Vol. 6, pp. 39–49.

Koch, Mathilde H. and Carl Voegtlin.

'16 Chemical Changes in the Central Nervous System as a Result of Restricted Vegetable Diet. Hygienic Lab. Bull., No. 103, 135 pp.

Lewis, M. R. and W. H. Lewis.

'15 Mitochondria (and other Cytoplasmic Structures) in Tissue Cultures. Amer. Jour. Anat., Vol. 17, pp. 339–401.

Löwschin, A. M.

'13 "Myelinformen" und Chondriosomen, Ber. deutsch. bot. Ges., Bd. 31, pp. 203–209.

Macallum, A. B.

'99 On the Cytology of Non-nucleated Organisms. Trans. Can. Inst., Vol. 6, pp. 1–70.

Mayer, Rathery and Schaeffer.

'14 Les granulations ou mitochondries de la cellule hépatique. Jour. de Phys. et de Path. gén., T. 16, pp. 607–622.

Meves, Fr.

'04 Über das Vorkommen von Mitochondrien bzw. Chondriomiten in Pflanzenzellen. Ber. d. deut. bot. Ges., Bd. 22, pp. 284–286.

Michaelis, L.

'99 Die vitale Färburg, eine Darstellungsmethode der Zellgranula. Arch. f. mikr. Anat., Bd. 55, pp. 558–575.

Moreau, F. and Madame F. Moreau.

'15 Sur le chondriome d'une algue vert, Coccomyxa solorinæ Chod. C. Rend. Soc. Biol., Paris, T. 78, pp. 729–730.

Nicholson, N. C.

'16 Morphological and microchemical Variations in the Mitochondria in the Cells of the Central Nervous System. Amer. Jour. Anat., Vol. 19, pp. 329–349.

Parker, T. Jeffery and William A. Haswell.

'97 A Text-book of Zoölogy. Vol. 1, Macmillan and Co., London, 779 pp.

Pensa, Antonio.

'14 Ancora a proposito di condriosomi e pigmento antocianico nelle cellule vegetali. Anat. Anz., Bd. 46, pp. 13–22.

Policard, A.

'12 Sensibilité des chondriosomes aux élévations de température. C. Rend. Soc. Biol., Paris, T. 72, pp. 228–229.

Regaud, Cl.

'08 Sur les mitochondries de l'epithélium seminal. C. Rend. Soc. Biol., Paris, T. 65, pp. 660–662.

'10 Étude sur la structure des tubes seminifères, etc. Arch. d'Anat. Micr., T. 11, pp. 291–433.

Rudolph, K.

'12 Chondriosomen und Chromatophoren. Beitrag zur Kritik der Chondriosomentheorien. Ber. d. Deutsch. Bot. Ges., Bd. 30, pp. 605–629.

Russo, Achille.

'12 Aumento dei granuli protoplasmatici, etc. Arch. f. Zellforsch., Bd. 8, pp. 203–215.

Sapëhin, A. A.

'15 Untersuchungen über die Individualität der Plastide. Arch. f. Zellforsch., Bd. 13, pp. 319–398.

Smirnow, A. E.

'06 Über die Mitochondrien und den Golgi'schen Bildungen Analoge Strukturen in einigen Zellen von Hyacinthus orientalis. Anat. Hefte, Bd. 32, pp. 146–153.

Ternetz, C.

'12 Beiträge zur Morphologie und Physiologie der Euglena gracilis Klebs. Jarhb. f. wis. Bot., Bd. 51, pp. 435–514.

Van der Stricht, Rene.

'11 Vitellogenese dans l'ovule de Chatte. Arch. de Biol., T. 26, pp. 365–481.

Van der Stricht, O.

'09 La structure de l'oeuf des Mammifères (Chauve-souris, Vesperugo noctula). Troisième partie. Mem. Cl. des sci. Acad. Belgique, 2e. Series, T. 11, 173 pp.

Zimmermann, A.

'93 Sammel-Referat aus den gesammtgebiete der Zellenlehre. Bot. Centralbl., Bd. 3, p. 215.